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Kuiper et al.

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(54) **ORPHAN RECEPTOR**

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(73) Assignee: **Karo Bio AB**, Huddinge (SE)

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 169 days.

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This patent is subject to a terminal disclaimer.

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(21) Appl. No.: **10/278,481**

Mosselman et al., "ERβ: identification and characterization of a novel human estrogen receptor", *FEBS Letters* 392, 1996, pp. 49-53.

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Vennstrom et al., "Isolation and Characterization of Chicken DNA Homologous to the Two Putative Oncogenes of Avian Erythroblastosis Virus", *Cell*, vol. 28, Jan. 1982, pp. 135-143.

(65) **Prior Publication Data**

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Related U.S. Application Data

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(63) Continuation of application No. 10/083,807, filed on Feb. 27, 2002, now abandoned, which is a continuation of application No. 09/333,057, filed on Jun. 14, 1999, now abandoned, which is a continuation of application No. 08/836,620, filed as application No. PCT/EP96/03933 on Sep. 9, 1996, now Pat. No. 5,958,710.

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Foreign Application Priority Data

Sep. 8, 1995 (GB) 9518272.1
Mar. 15, 1996 (GB) 9605550.4
Apr. 11, 1996 (GB) 9607532.0
May 8, 1996 (GB) 9609576.5

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(51) **Int. Cl.**

C12N 15/12 (2006.01)
C07K 14/705 (2006.01)
C12P 21/06 (2006.01)

Bishop, "Oncogenes as hormone receptors", *Nature*, vol. 321, May 1986.

(52) **U.S. Cl.** **435/69.1**; 536/23.5; 435/320.1; 435/325; 530/350

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(58) **Field of Classification Search** 536/23.5; 530/350; 435/7.21, 6, 69.1, 320.1, 325
See application file for complete search history.

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(57) **ABSTRACT**

This invention relates to a novel estrogen receptor-related nuclear receptor, hereinafter termed "ERβ" having the amino acid sequence of FIG. 1, 13A or 14A or substantially the same amino acid sequence as the amino acid sequence shown in FIG. 1, 13A or 13B or an amino acid sequence functionally similar to that sequence. The invention also relates to DNA sequences encoding the receptor. The receptor may be useful in isolating molecules for the treatment of disorders such as prostate cancer, benign prostatic hyperplasia, osteoporosis or cardiovascular disorders and in the testing of substances for estrogenic and other hormonal effects.

13 Claims, 17 Drawing Sheets

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FIG. 1

ggaattcCGGGGAGCTGGCCCAGGGGAGCGGCTGGTGCTGCCACTGGCATCCCTAGGC 60
 ACCCAGGTCTGCAATAAAGTCTGGCAGCCACTGCATGGCTGAGCGACAACCAGTGGCTGG 120
 GAGTCCGGCTCTGTGGCTGAGGAAAGCACCTGTCTGCATTTAGAGAATGCAAAATAGAGA 180
 ATGTTTACCTGCCAGTCATTACATCTGAGTCCCATGAGTCTCTGAGAACATAATGTCCAT 240
 CTGTACCTCTTCTCACAAGGAGTTTTCTCAGCTGCGACCCCTCTGAAGACATGGAGATCAA 300
 AACTCACCGTCGAGCCTTAGTTCCTGTCTCCTATAACTGTAGCCAGTCCATCCTACCC 360
 CTGGAGCACGGCCCCATCTACATCCCTTCCTCCTACGTAGACAACCGCCATGAGTATTCA 420
 GCTATGACATTCTACAGTCTGTGTGATGAACTACAGTGTCCCGGCAGCACCAGTAAC 480
M T F Y S P A V M N Y S V P G S T S N
 CTGGACGGTGGGCCTGTCCGACTGAGCACAAGCCCAAATGTGCTATGGCCAACCTTCTGGG 540
 L D G G P V R L S T S P N V L W P T S G
 CACCTGTCTCCTTTAGCGACCCATTGCCAATCATCGCTCCTCTATGCAGAACCTCAAAAG 600
 H L S P L A T H C Q S S L L Y A E P Q K
 AGTCCTTGGTGTGAAGCAAGATCACTAGAGCACACCTTACCTGTAAACAGAGAGACTG 660
 S P W C E A R S L E H T L P V N R E T L
 AAGAGGAAGCTTAGTGGGAGCAGTTGTGCCAGCCCTGTACTAGTCCAAACGCAAAGAGG 720
 K R K L S G S S C A S P V T S P N A K R
 GATGCTCACTTCTGCCCCGTCTGCAGCGATTATGCATCTGGGTATCATTACGGCGTTTGG 780
 D A H F C P V C S D Y A S G Y H Y G V W
 TCATGTGAAGGATGTAAGGCCTTTTAAAGAAGCATTCAAGGACATAATGATTATATC 840
S C E G C K A F F K R S I Q G H N D Y I
 TGTCCAGCCACGAATCAGTGTACCATAGACAAGAACCGCGTAAAAGCTGCCAGGCCTGC 900
C P A T N Q C T I D K N R R K S C Q A C
 CGACTTCGCAAGTGTATGAAGTAGGAATGGTCAAGTGTGGATCCAGGAGAGAACGGTGT 960
R L R K C Y E V G M V K C G S R R E R C
 GGGTACCGTATAGTGGGAGGCAGAGAAGTCTAGCGAGCAGGTACACTGCCTGAGCAA 1020
 G Y R I V R R Q R S S S E Q V H C L S K
GCCAAGAGAAACGGTGGGCATGCACCCCGGGTGAAGGAGCTACTGCTGAGCACCTTGAGT 1080
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 CCAGAGCAACTGGTGTCCACCTCCTGGAAGCTGAACCACCCAATGTGCTGGTGAGCCGT 1140
 P E Q L V L T L L E A E P P N V L V S R
 CCCAGCATGCCCTTCACCGAGGCCTCCATGATGATGTCCCTCACTAAGCTGGCCGACAAG 1200
 P S M P F T E A S M M M S L T K L A D K
GAAGTGGTGCACATGATTGGCTGGGCCAAGAAAATCCCTGGCTTTGTGGAGCTCAGCCTG 1260
 E L V H M I G W A K K I P G F V E L S L
 TTGGACCAAGTCCGGCTCTTAGAAAAGCTGCTGGATGGAGGTGCTAATGGTGGGACTGATG 1320
 L D Q V R L L E S C W M E V L M V G L M
 TGGCGCTCCATCGACCACCCCGGCAAGCTCATTTTCGCTCCCGACCTCGTTCTGGACAGG 1380
 W R S I D H P G K L I F A P D L V L D R
 GATGAGGGGAAGTGCCTAGAAAGGATCTGGAATCTTTGACATGCTCCTGGCGACGACG 1440
 D E G K C V E G I L E I F D M L L A T T
 TCAAGGTTCCGTGAGTTAAACTCCAGCACAAGGAGTATCTCTGTGTGAAGCCATGATC 1500
 S R F R E L K L Q H K E Y L C V K A M I
 CTCCTCAACTCCAGTATGTACCCCTTGGCTTCTGCAAACCAGGAGGCAGAAAAGTAGCCGG 1560
 L L N S S M Y P L A S A N Q E A E S S R
 AAGCTGACACACCTACTGAACCGGGTGACAGATGCCCTGGTCTGGGTGATTGCGAAGAGT 1620
 K L T H L L N A V T D A L V W V I A K S
 GGTATCTCCTCCCAGCAGCAGTCAGTCCGACTGGCCAACCTCCTGATGCTTCTTTCTCAC 1680
 G I S S Q Q Q S V R L A N L L M L L S H
 GTCAGGCACATCAGTAACAAGGGCATGGAACATCTGCTCAGCATGAAGTGCAAAAATGTG 1740
 V R H I S N K G M E H L L S M K C K N V
 GTCCCGGTGTATGACCTGCTGCTGGAGATGCTGAATGCTCACACGCTTCGAGGGTACAAG 1800
 V P V Y D L L L E M L N A H T L R G Y K
 TCCTCAATCTCGGGTCTGAGTGCAGCTCAACAGAGGACAGTAAGAACAAAGAGAGCTCC 1860
 S S I S G S E C S S T E D S K N K E S S
 CAGAACCTACAGTCTCAGTGTGGCCAGGCCTGAGGCGGACAGACTACAGAGATGGTCAA 1920
 Q N L Q S Q *
 AAGTGAACATGTACCCTAGCATCTGGGGTTCCTCTTAGGGCTGCCTTGGTTACGCACC 1980
 CCTTACCCCACTGCCTTCCCAGGAGTCAGGGTGGTTGTGTGGCGGTGTTCCTCATACC 2040
 AGGATGTACCACCGAATGCCAAGTCTAACTTGTATAGCCTTGAAGGCTCTCGGTGTACT 2100
 TACTTCTGTCTCCTTGCCCACTTGGAAACATCTGAAAGGTTCTGGAATAAAGGTCAA 2160
 GTCTGATTTGGAAGGATTGTCTTAGTCAGGAAAAGGAATATGGCATGTGACACAGCTAT 2220
 AAGAAATGGACTGTAGGACTGTGTGGCCATAAAATCAACCTTTGGATGGCGTCTCTAGA 2280
 CCACTTGATTGTAGGATTGAAAACCATTTGACAATCAGCTCATTTTCGATTCTTCGCTC 2340
 ACGGGTCTGTGAGGACTCATTAAATGTATGGTTATTCTATCAAAGACCAGAAAAGATAGT 2400
 GCAAGCTTAGATGTACCTTGTTCCTCCTCCAGACCCCTGGGTTACATCCTTAGAGCCTG 2460
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FIG. 2A

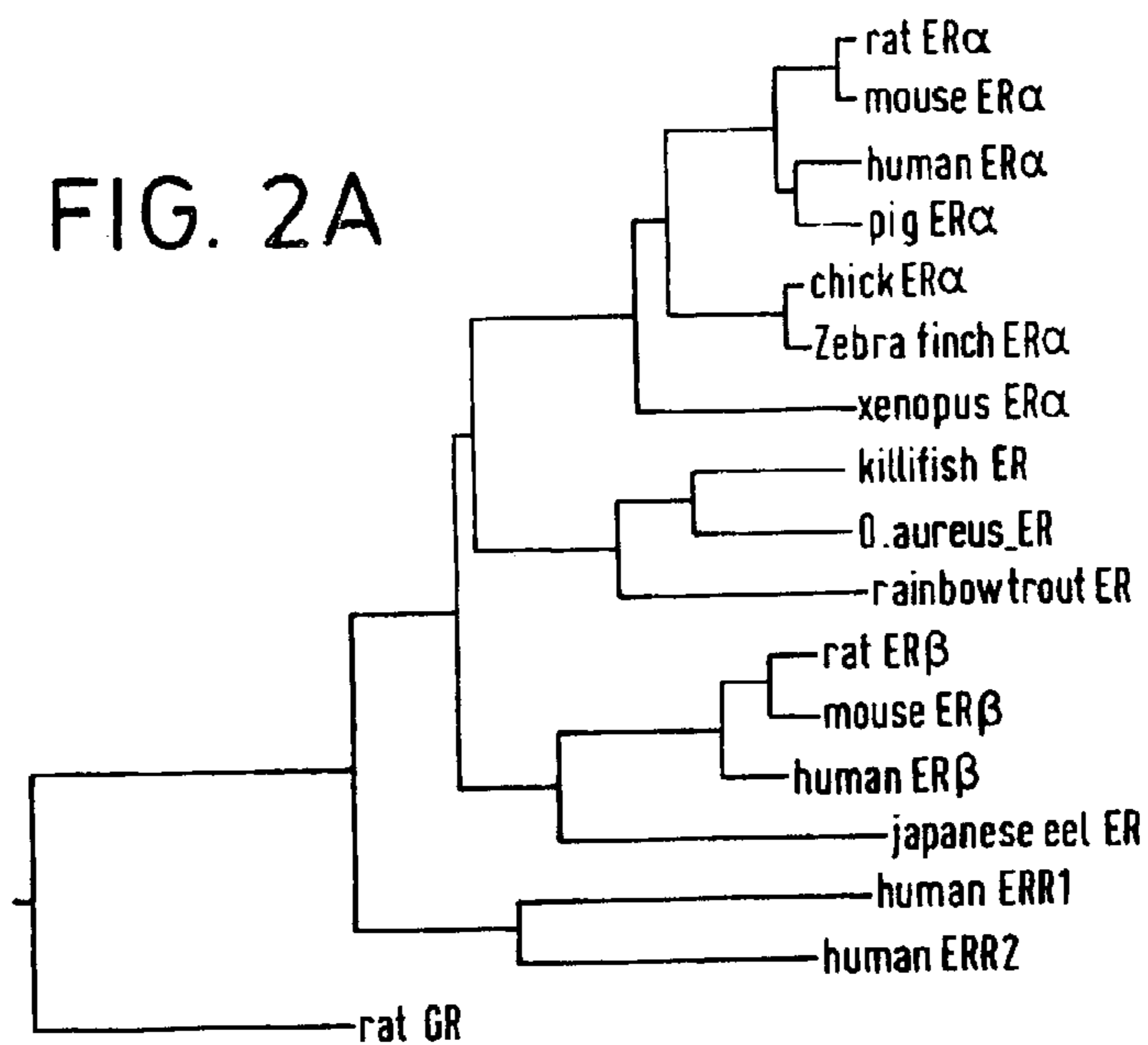
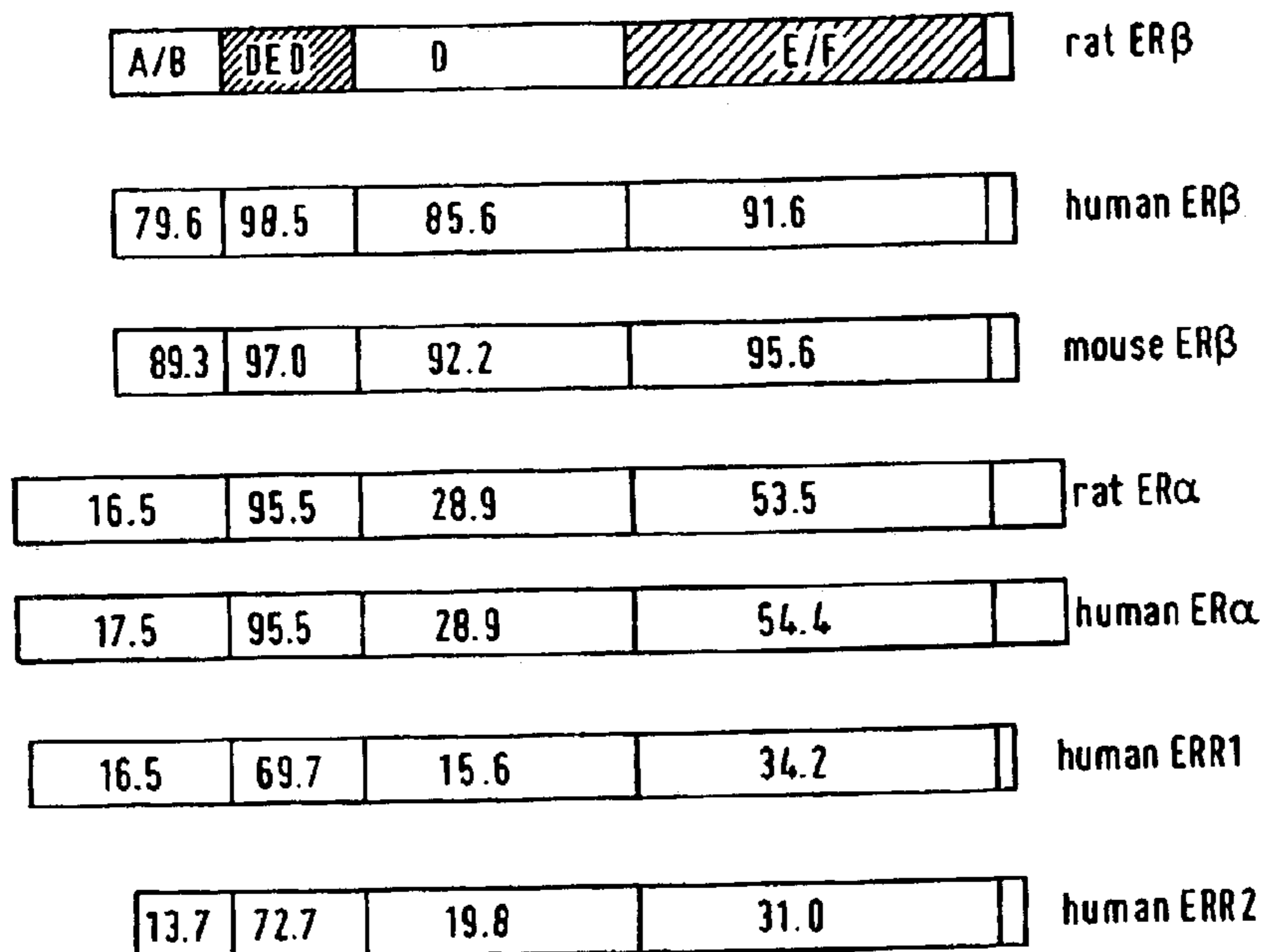


FIG. 2B ALIGNMENT OF ERβ TO OTHER ESTROGEN RECEPTORS



Ligandbinding domain

ELVHMIGWAKKIPGFVLSLLDQVRLLESCWMEVLMVGLMWRSIDHPGKL	ERβ rat
.....N...RV...GD.N.H...H...CA.L.I...I...V...ME... ..	ER rat
.....N...RV...GD.N.H...H...CA.L.I...I...V...ME... ..	ER mouse
.....N...RV...D.T.H...H...CA.L.I...I...V...ME... ..	ER human
IFAPDLVLDREDEGKCVEGILEIFDMLLATTSRFRLELQHKELYLCVKAMI	ERβ rat
L...N.L...NQ...M.V...S...MMN...GE.FV.L.SI..	ER rat
L...N.L...NQ...M.V...S...MMN...GE.FV.L.SI..	ER mouse
L...N.L...NQ...M.V...S...MMN...GE.FV.L.SI..	ER human
LLNSSMYP-LASANQEAESSRKLTHLLNNAVTDALVWVIAKSGISSQQQSSV	ERβ rat
...GV.TF.S.TLKSL.EKDHHRV.DKIN.T.IHLM...A.LTL...HR	ER rat
...GV.TF.S.TLKSL.EKDHHRV.DKI..T.IHLM...A.LTL...HR	ER mouse
...GV.TF.S.TLKSL.EKDHHRV.DKI..T.IHLM...A.LTL...HQ	ER human
RLANLLMLLSHVRI SNKGMELLSMKCKNVVPVYD ^U LLLEMLNAHTLRG-	ERβ rat
...Q...LI...I...M...Y.N...L...D...R.HAP	ER rat
...Q...LI...I...M...Y.N...L...D...R.HAP	ER mouse
...Q...LI...I...M...Y...L...D...R.HAP	ER human
-YKSSISGSECSSTE-D SKNKES SQNLQS- - - - - Q	ERβ rat
ASRMGV PPE.P.QS QLTTSSST.AHS...TY YIPPEAEGFPNTI	ER rat
ASRMGV PPE.P.Q.QLATTSST.AHS...TY YIPPEAEGFPNTI	ER mouse
TSRGGGA.VE.TDQSHLATAGST...HS...KYYITGEAEGFPATV	ER human

TAF-2

FIG. 2C

DNA-binding domain

C	P	V	C	S	D	Y	A	S	G	Y	H	Y	G	V	W	S	C	E	G	C	K	A	F	F	K	R	S	I	Q	G	H	N	D	Y	I	C	P	A	T	N	O	C	T	I	D	K	N	R	R	K	S	C	O	A	C	R	L	R	K	C	Y	E	V	G	M	ER _β rat
.	A	.	.	.	N	ER rat						
.	A	.	.	.	N	ER mouse						
.	A	.	.	.	N	ER human					

FIG. 2D



FIG. 3A



FIG. 3B

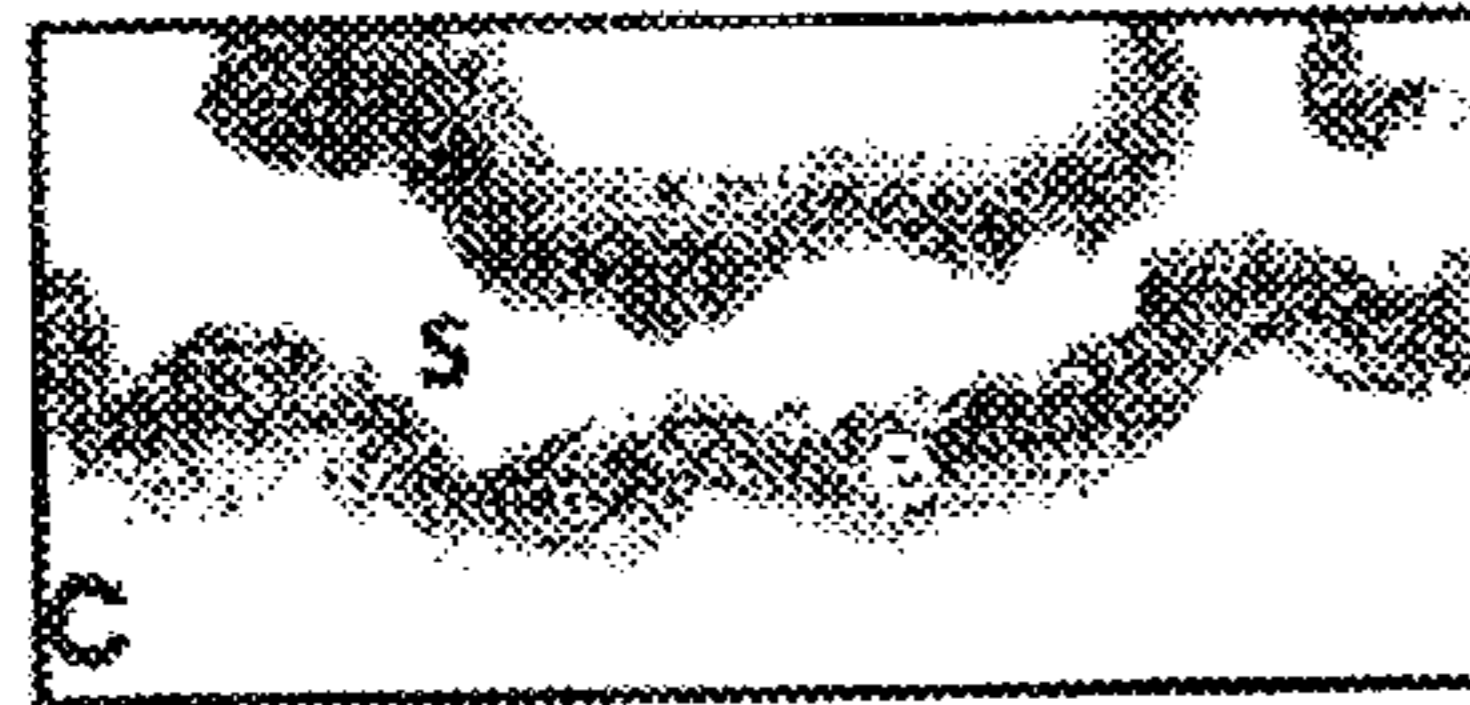


FIG. 3C

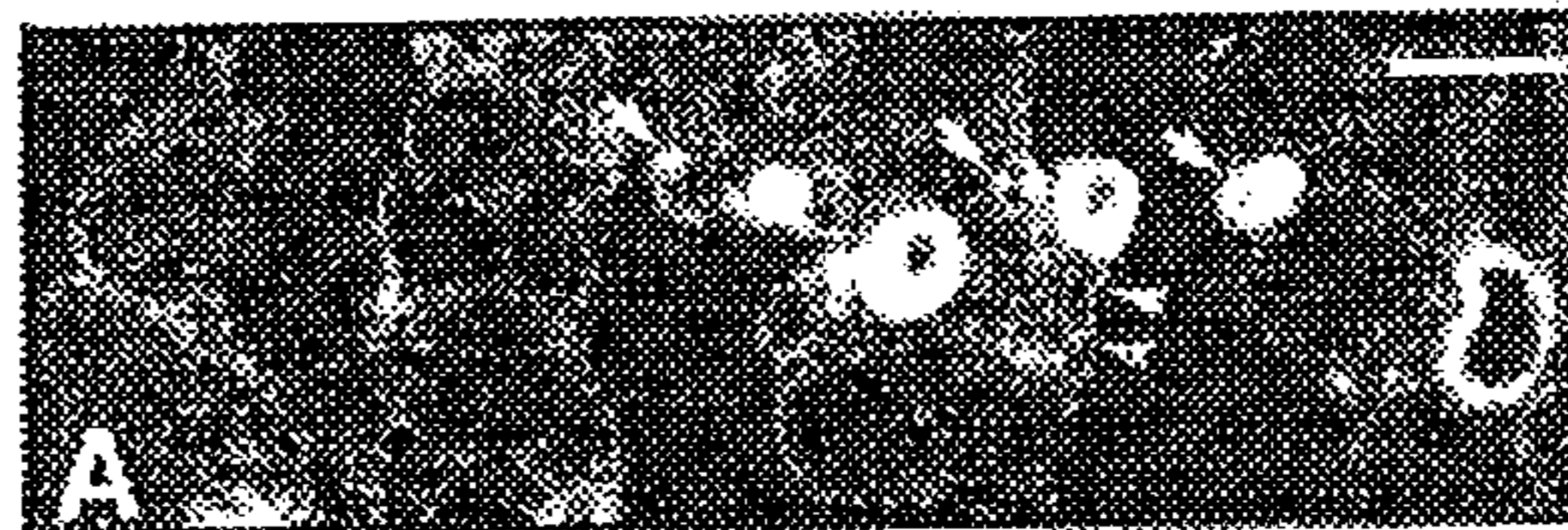


FIG. 4A

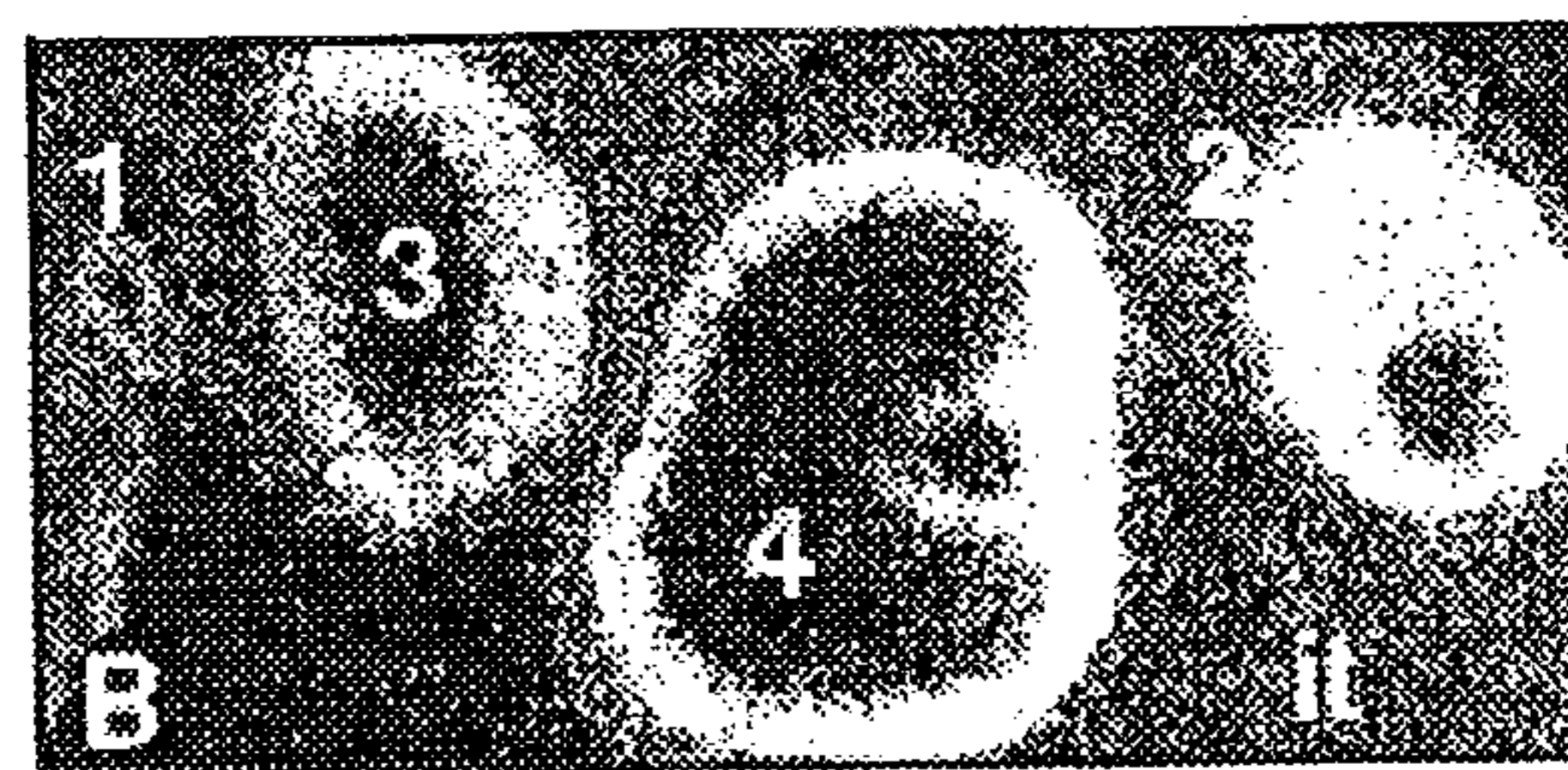


FIG. 4B

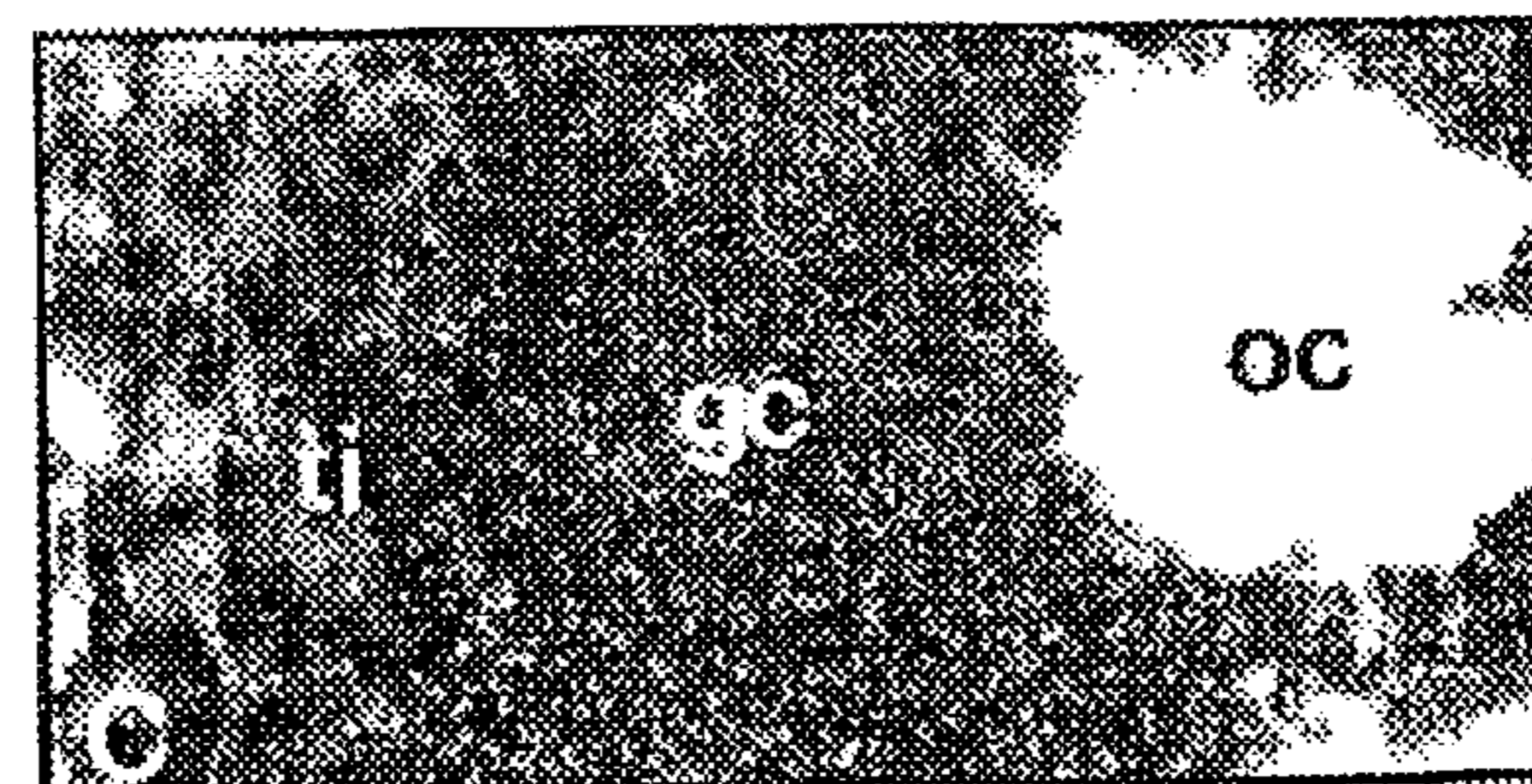
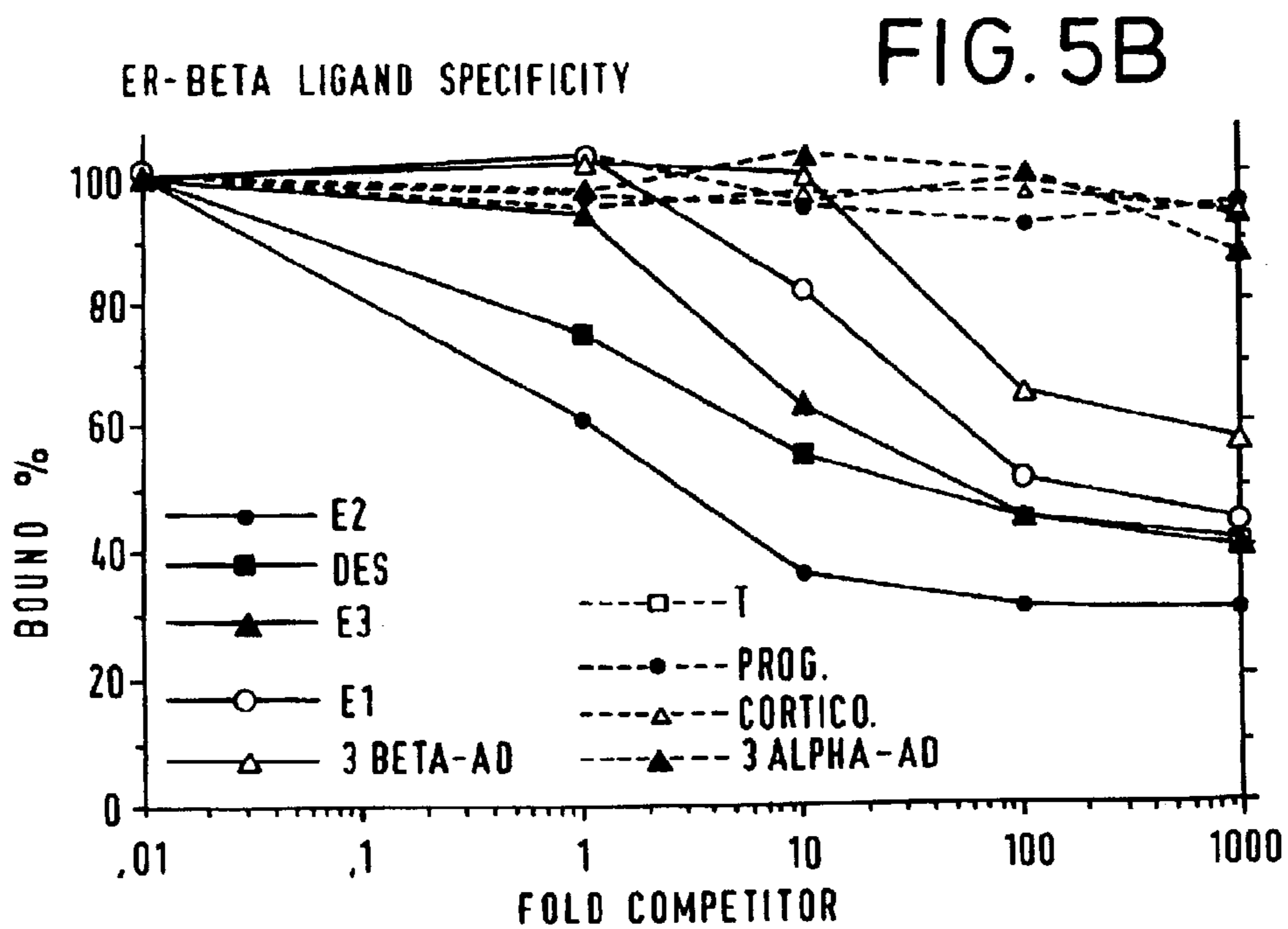
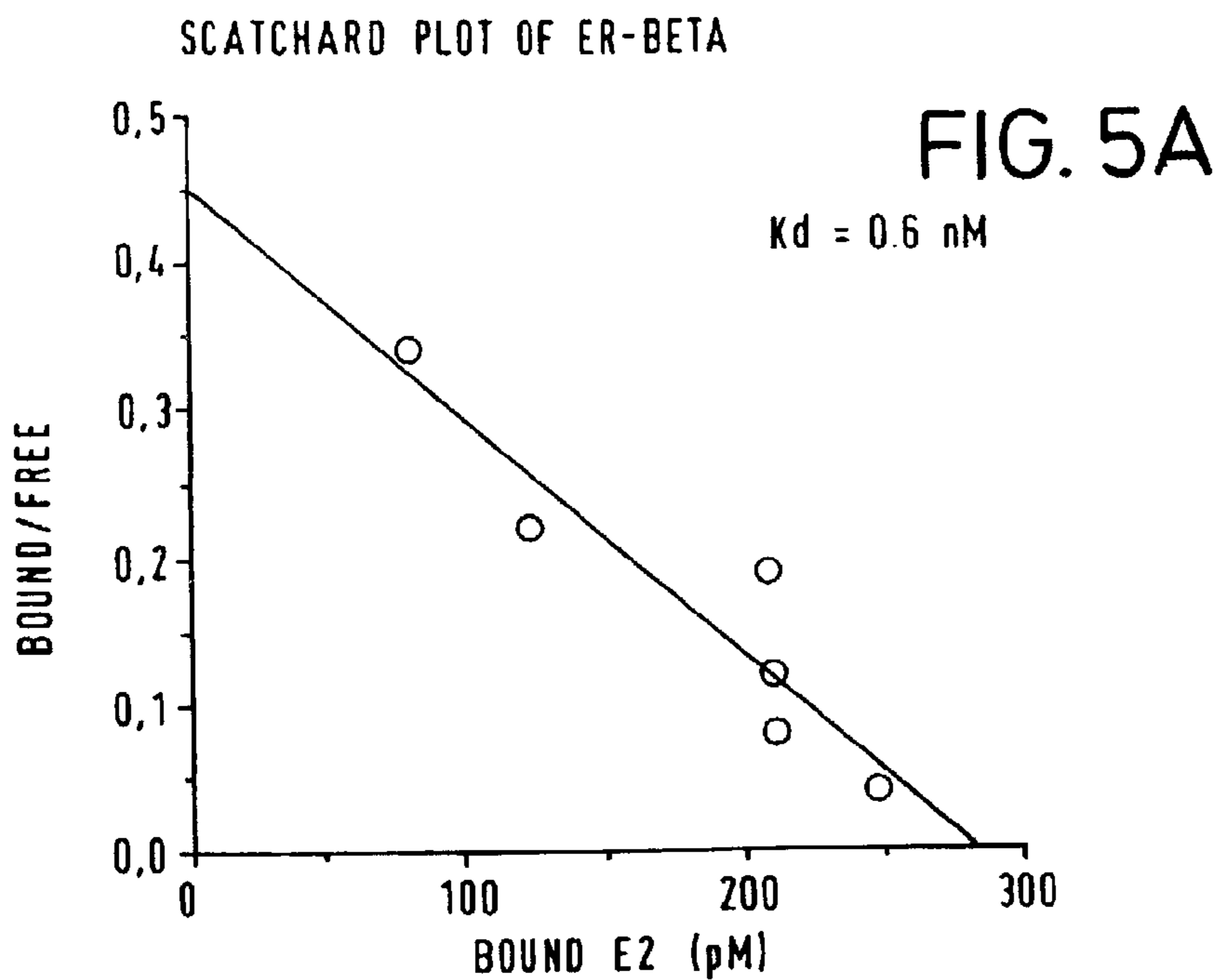


FIG. 4C



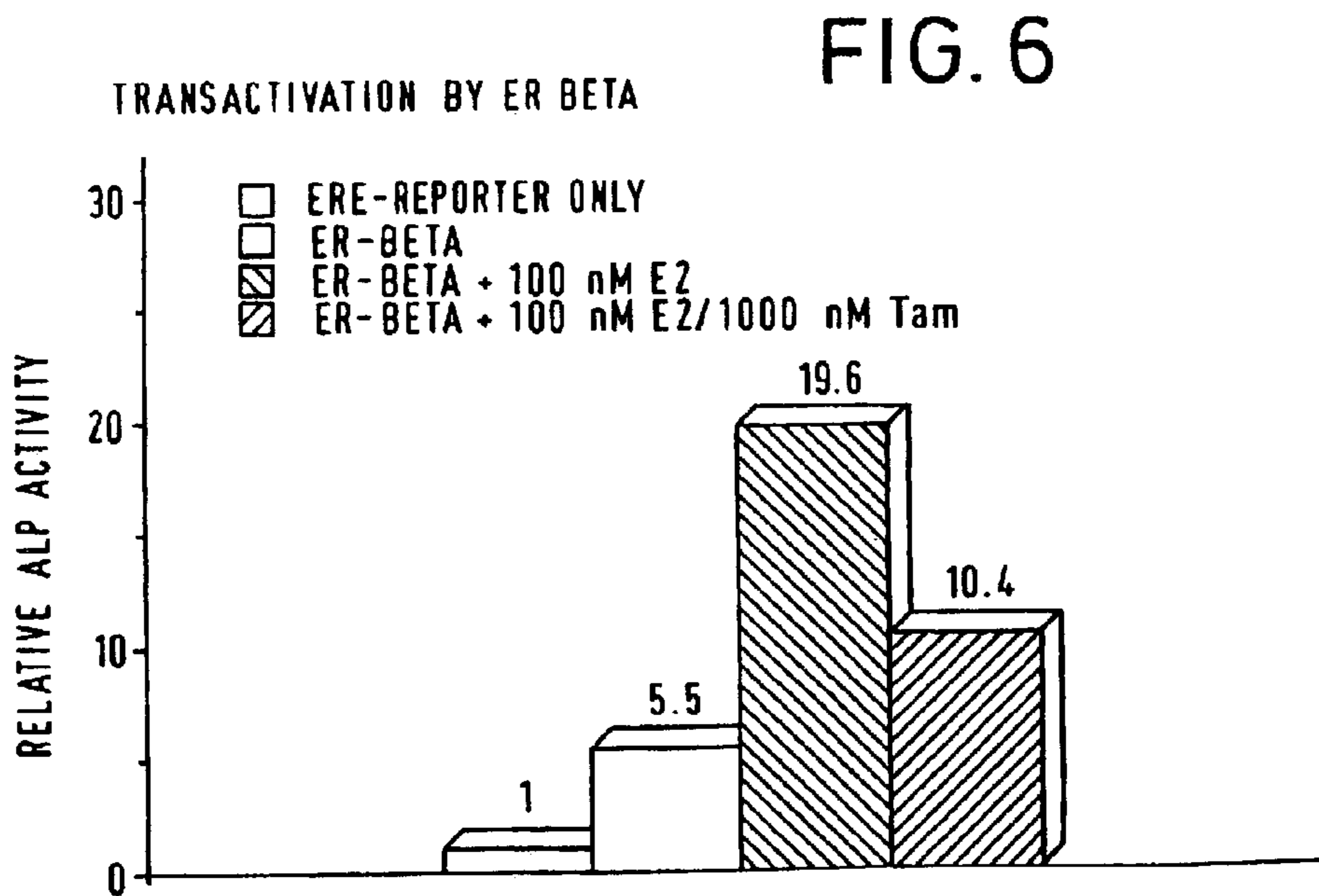
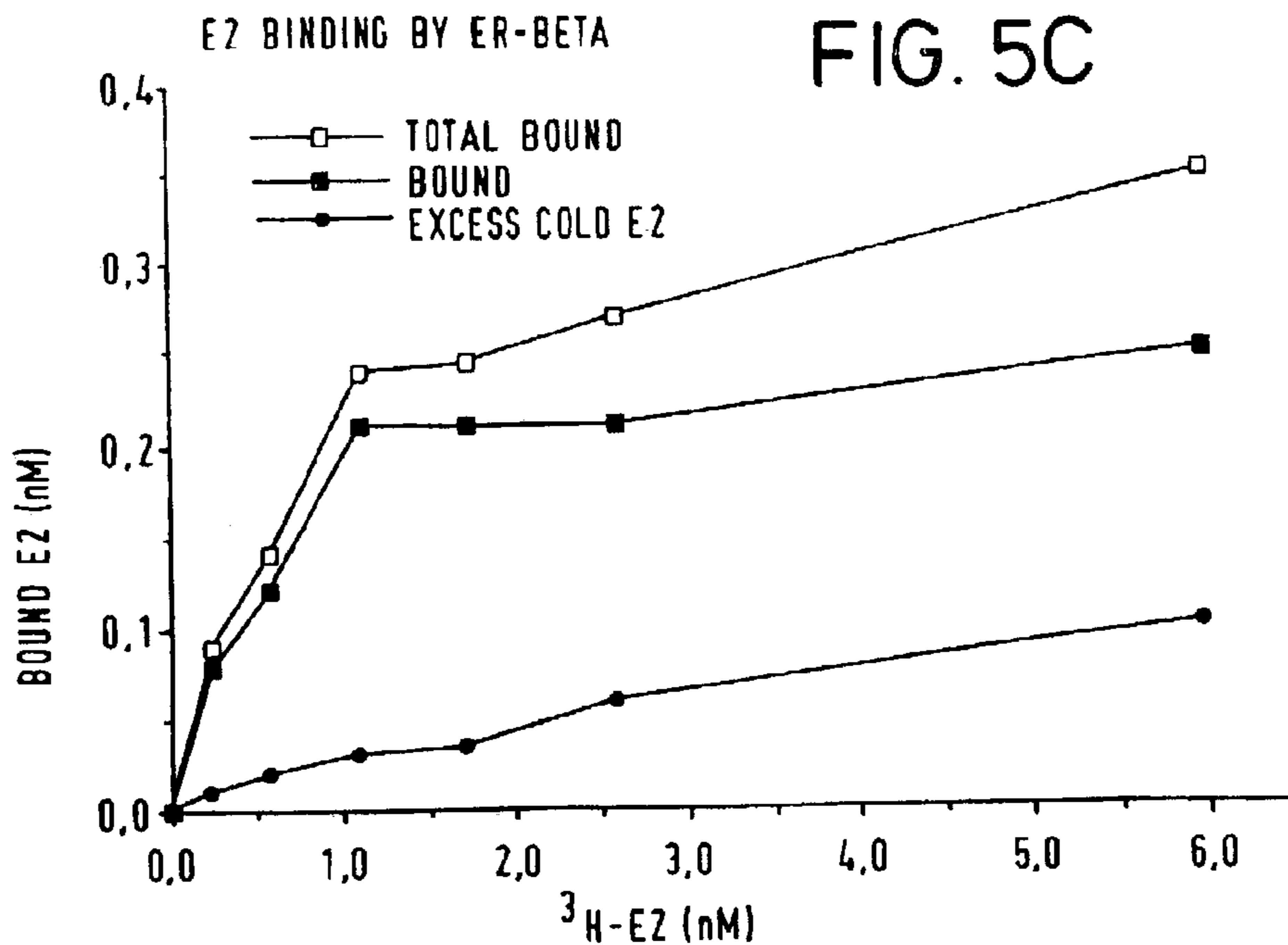


FIG. 7

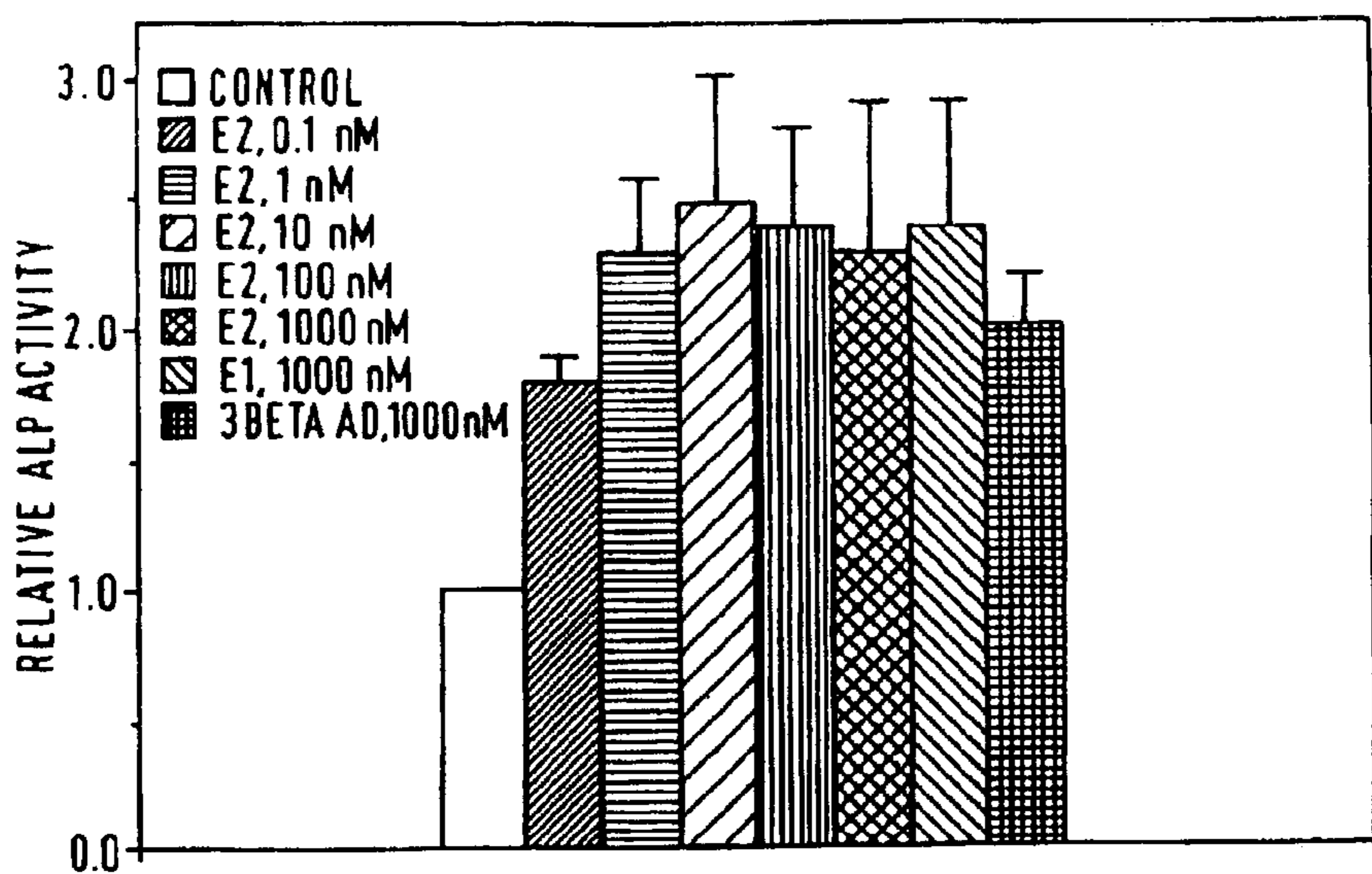
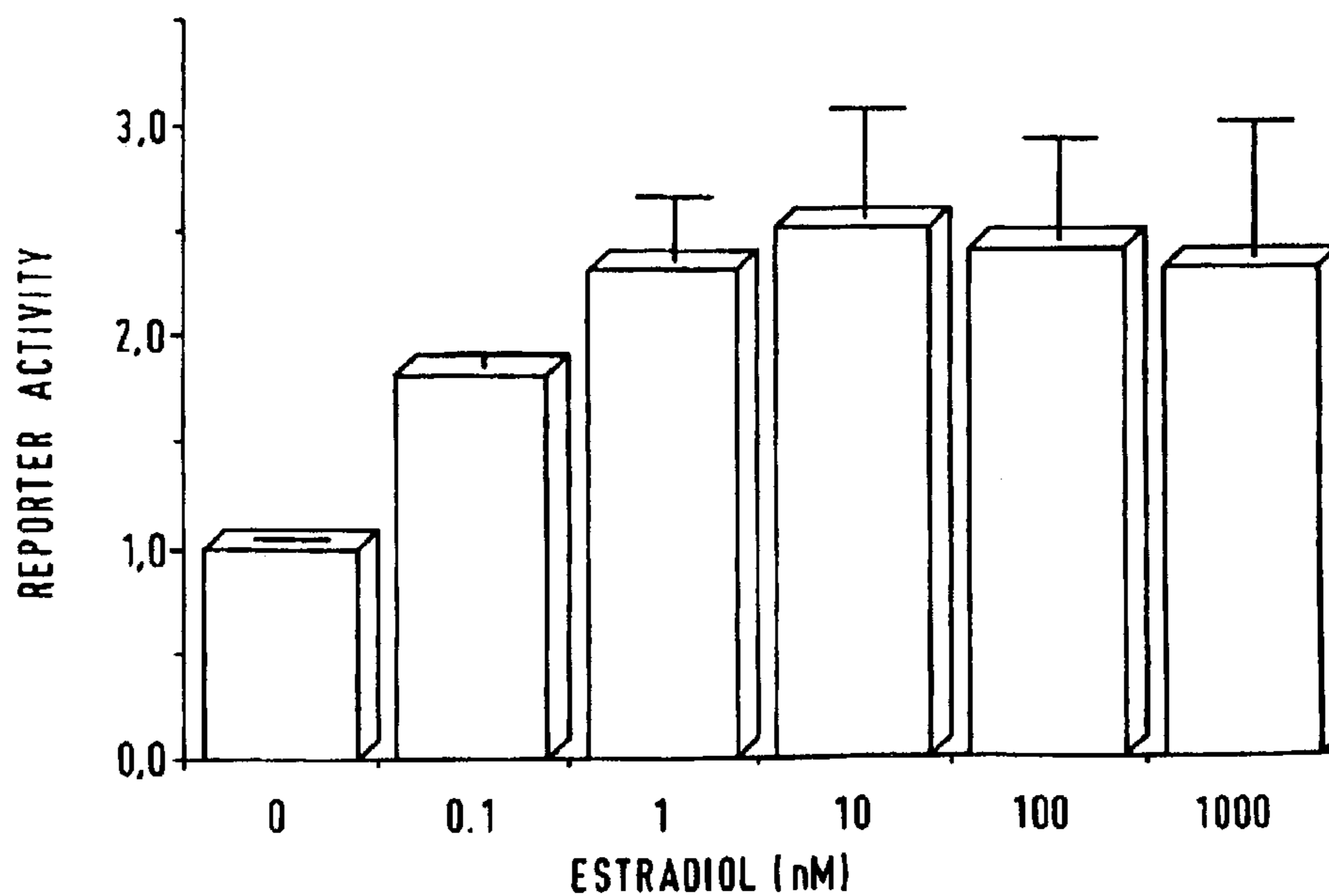


FIG. 7A

E2 STIMULATED TRANSACTIVATION



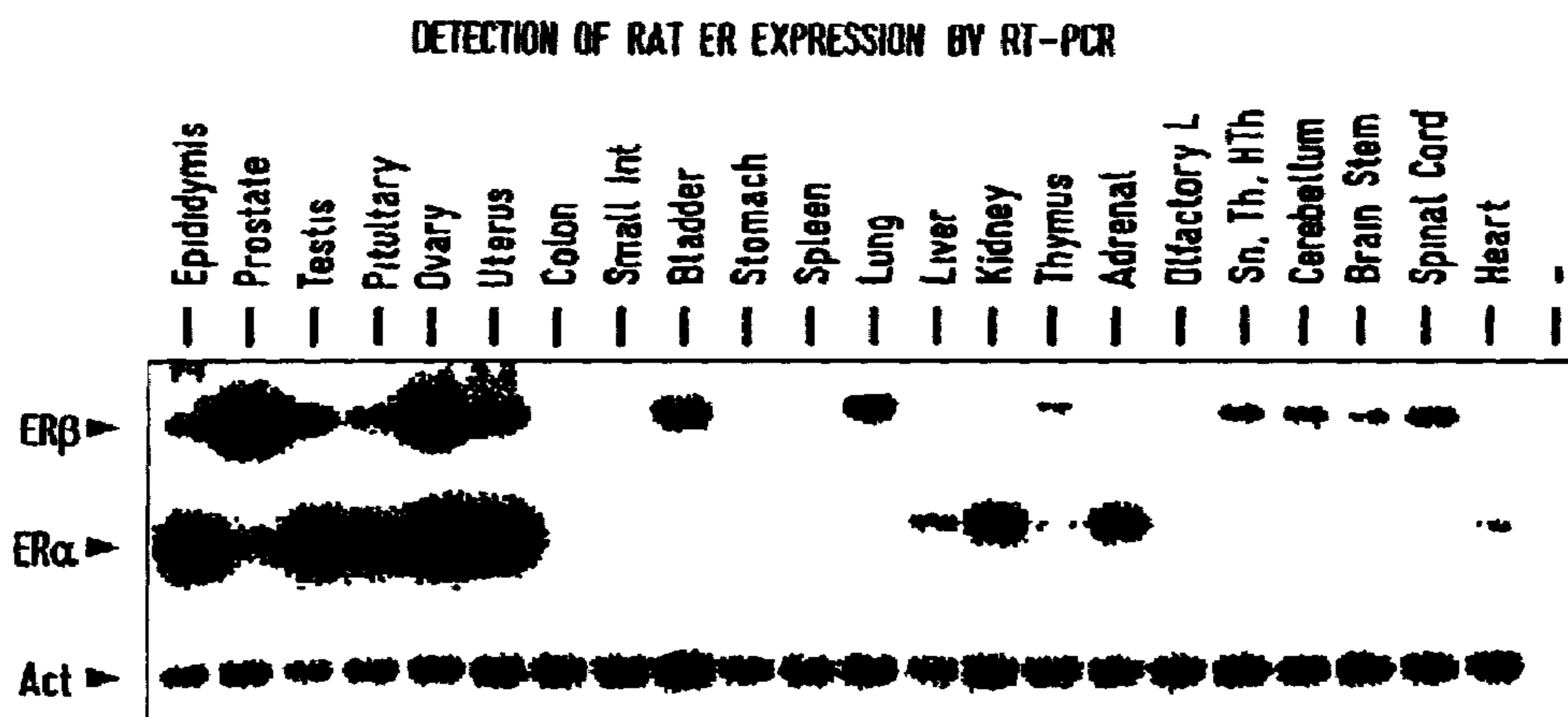


FIG. 8

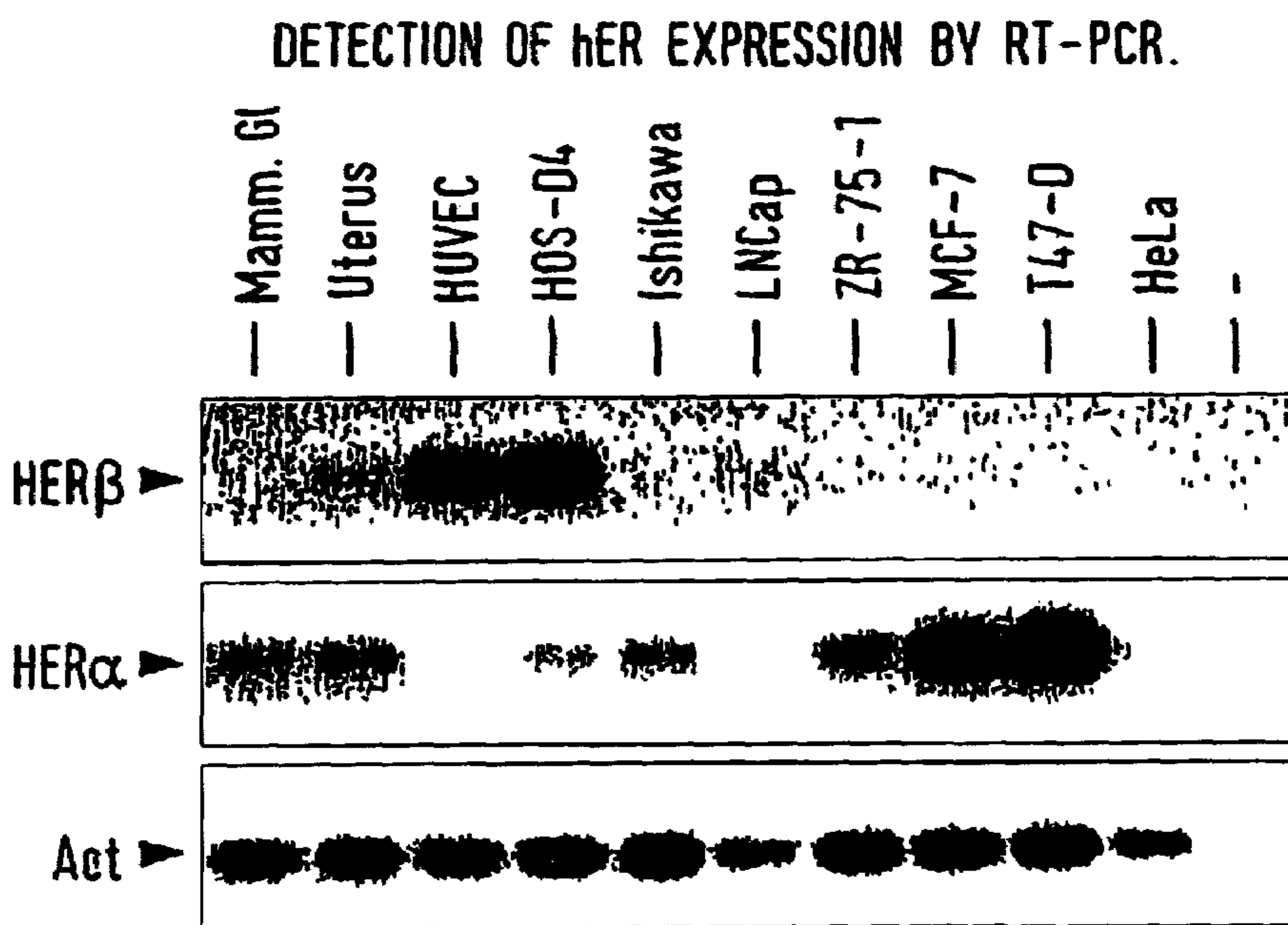


FIG. 9

FIG. 10A

HILL PLOT COMPARING hER α AND rER β .

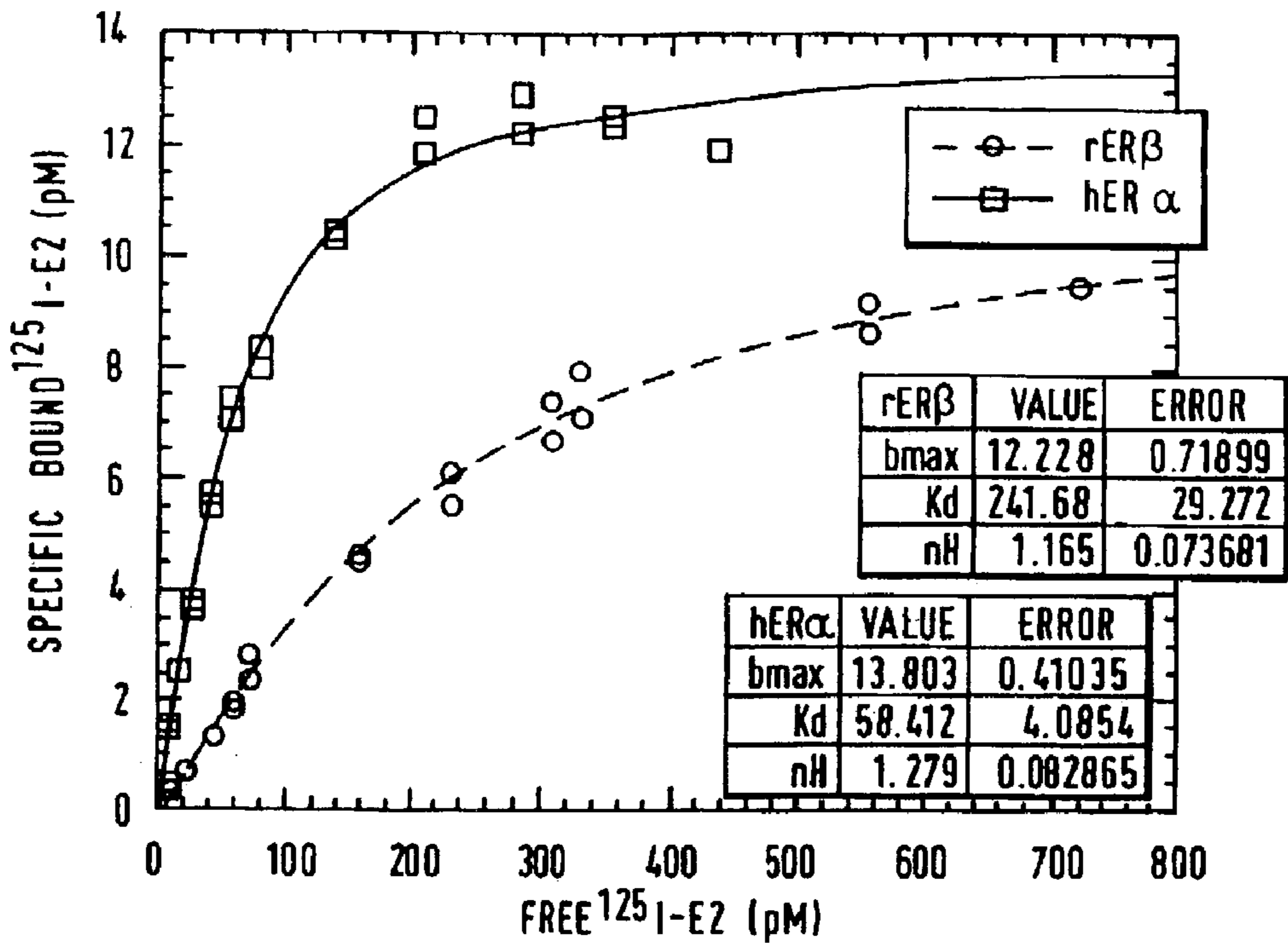


FIG. 10B

SCATCHARD PLOT COMPARING hER α AND rER β .

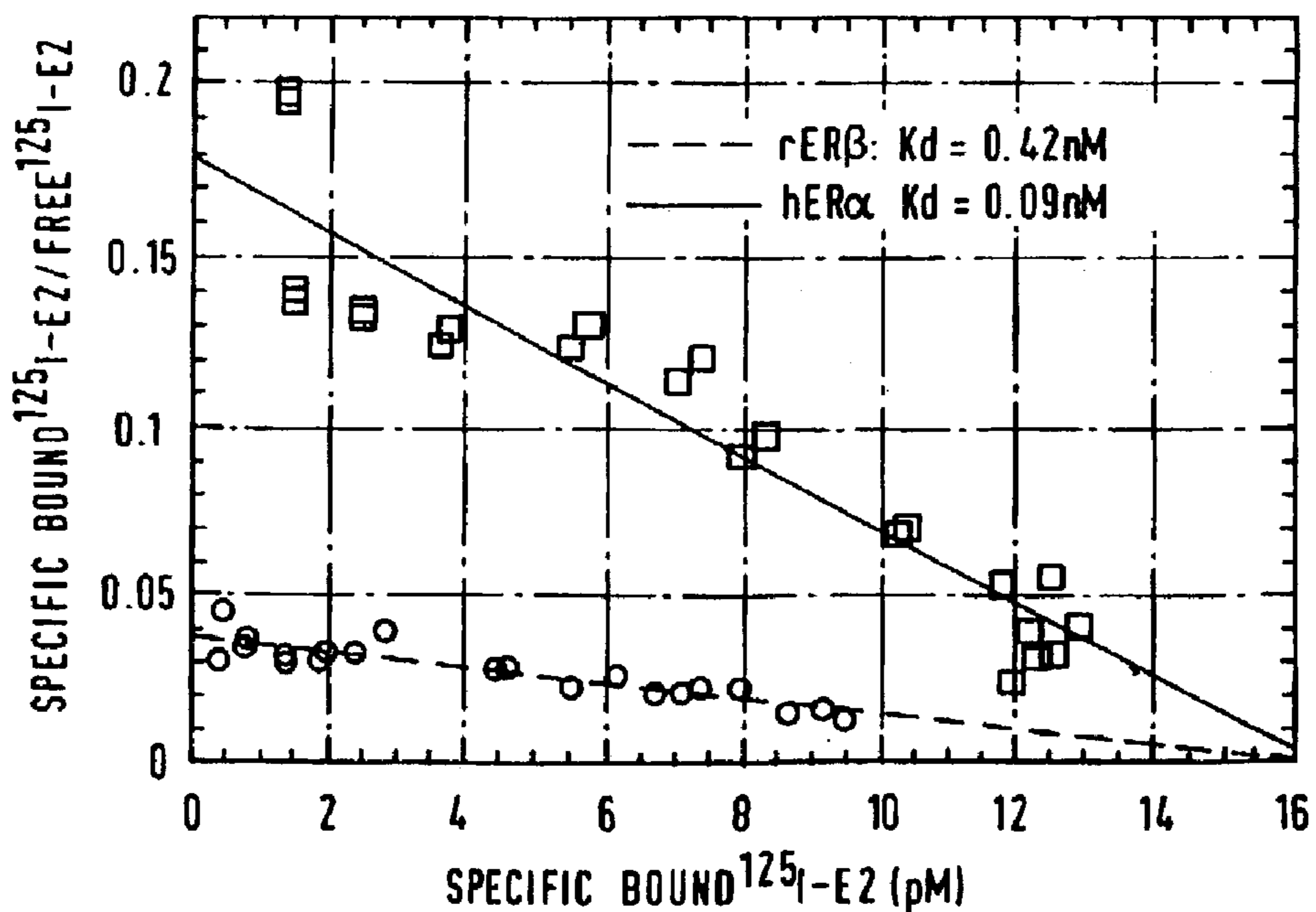


FIG.11A

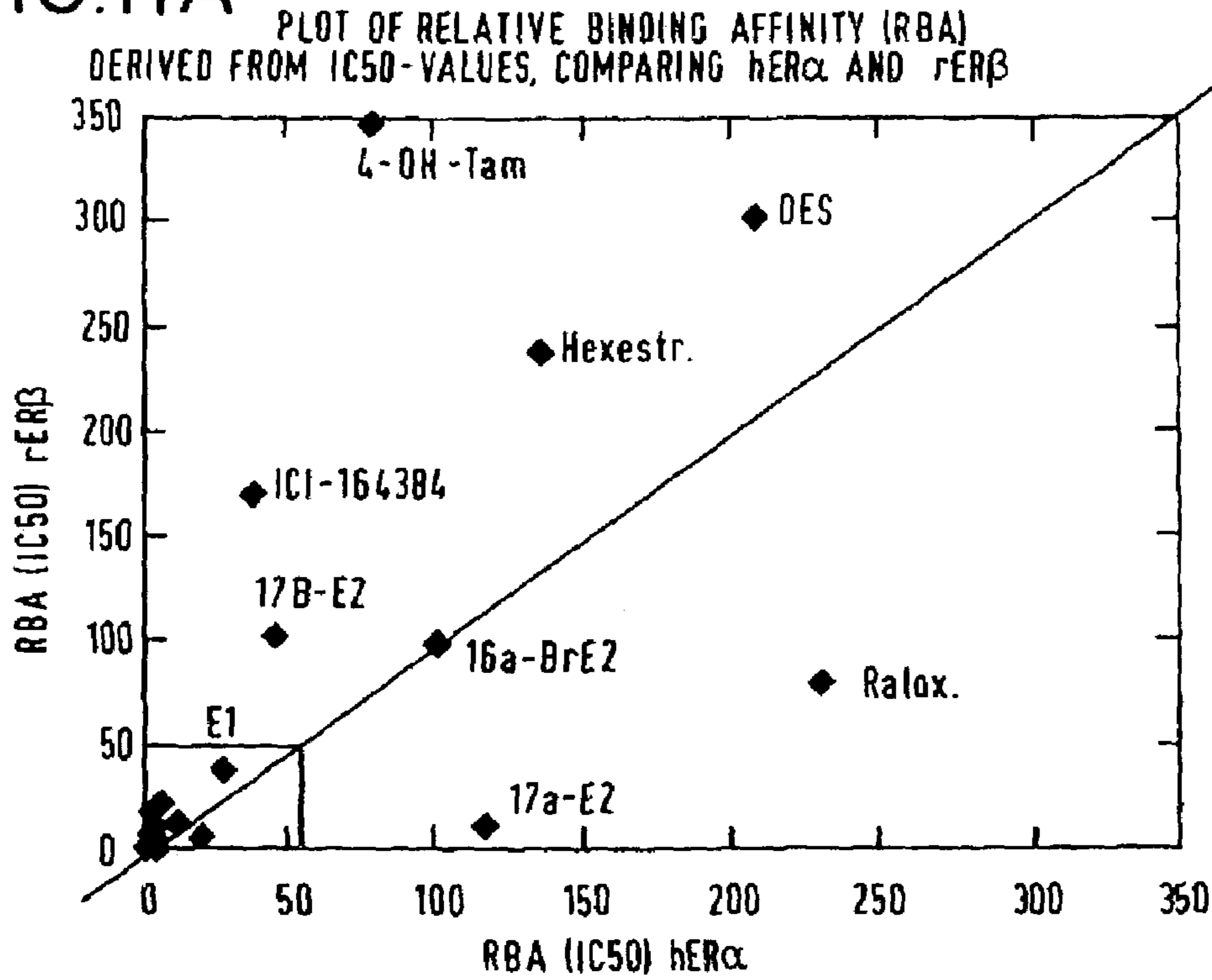
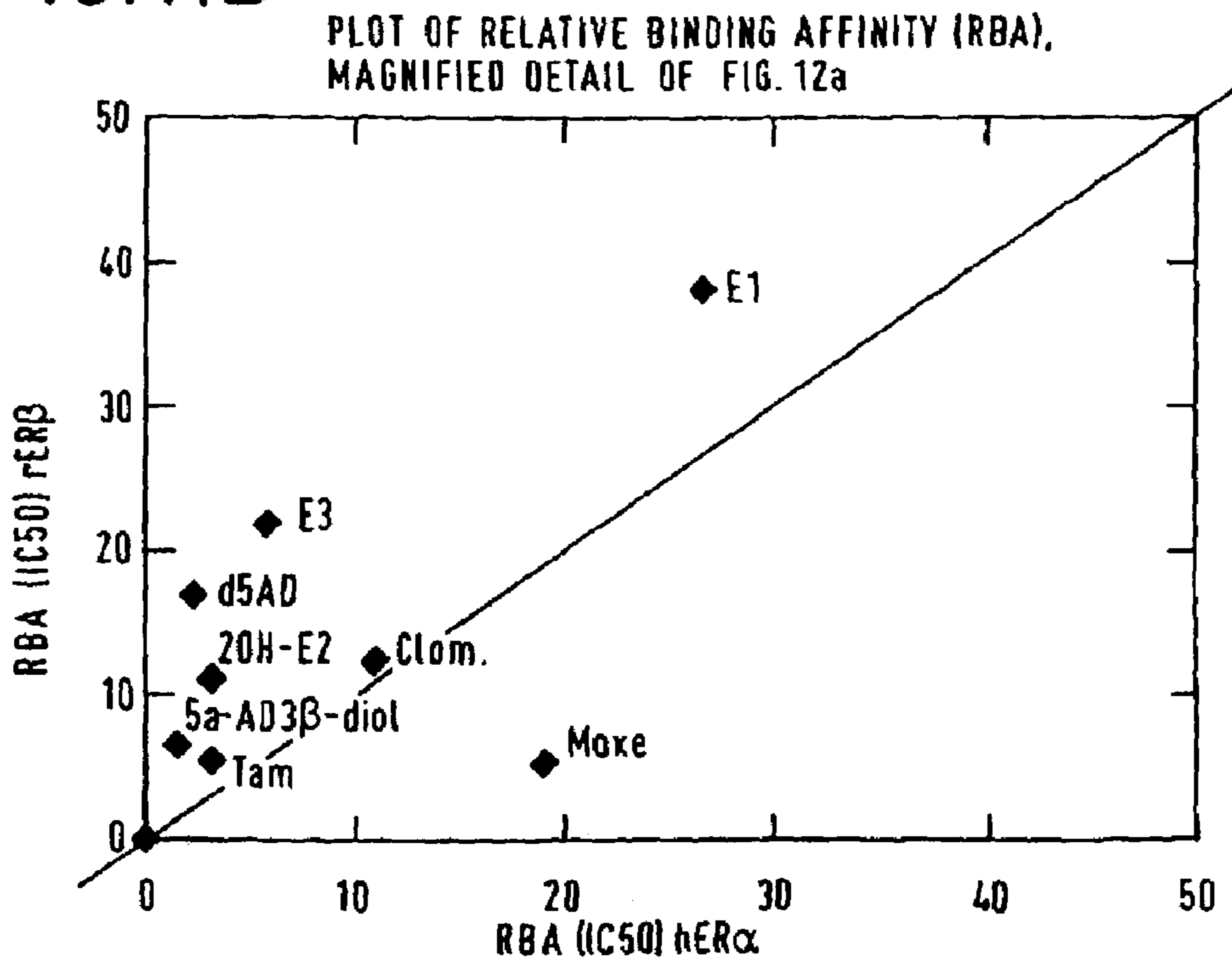


FIG.11B



1 MTFYSPAVMN YSIPSNVTNL EGGPGRQTTS PNVLWPTPGH LSPLVVHRQL
 51 SHLYAEPQKS PWCEARSLEH TLPVNRETLK RKVSGNRCAS PVTGPGSKRD
 101 AHFCAVCS DY ASGYHYGVWS CEGCKAFFKR SIQGHNDYIC PATNQCTIDK
 151 NRRKSCQACR LRKCYEVGMV KCGSRRERCG YRLVRRQRSA DEQLHCAGKA
 201 KRSGGHAPRV RELLLDALSP EQLVLTLEA EPPHVLISRP SAPFTEASMM
 251 MSLTKLADKE LVHMISWAKK IPGFVELSLF DQVRLLESCW MEVLMGLMW
 301 RSIDHPGKLI FAPDLVLD RD EGKCVEGILE IFDMLLATT S RFRELKLQHK
 351 EYLCVKAMIL LNSSMYPLVT ATQDADSSRK LAHLLNAVTD ALVWVIKSG
 401 ISSOQQSMRL ANLLMLLSHV RHASNKGMEH LLNMKCKNVV PVDLLEML
 451 NAHVLRGCKS SITGSECS PA EDSKSKEGSQ NLQSQ*

FIG. 13A

MAFYSPAVMNY SVPSSTGNLEGGPVRQTASPNVLWPTS GH 40
 LSPLATHCQSSLLYAEPQKSPWCEARSLEHTLPVNRETLK 80
 RKLGGSGCASPV TSPSTKRDAHFCAVCS DYASGYHYGVWS 120
 CEGCKAFFKR SIQGHNDYICPATNQCTIDKNRRKNCQACR 160
 LRKCYEVGMV KCGSRRERCGYRIVRRQRSASEQVHCLNKA 200
 KR TSGHTPRVKELLLNSLSPEQLVLTLEAEPNVLVSRP 240
 SMPFTEASMM MSLTKLADKELVHMIGWAKKIPGFVELSLL 280
 DQVRLLESCWMEVLMVGLMWRSIDHPGKLIFAPDLVLD RD 320
 EGKCVEGILEIFDMLLATTARFRELKLQHK EYLCVKAMIL 360
 LNSSMYHLATASQEAESSRKLTHLLNAVTDALVWVISKSR 400
 ISSOQQSVRLANLLMLLSHV RHASNKGMEHLLSMKCKNVV 440
 PVDLLEMLNAHTLRGYKSSISGSGCCSTEDSKSKEGSQ 480
 NLQSQ. 486

FIG. 14A

1 CTATGACATT CTACAGTCCT GCTGTGATGA ATTACAGCAT TCCCAGCAAT
51 GTCACATACT TGGAAGGTGG GCCTGGTCGG CAGACCACAA GCCCAAATGT
101 GTTGTGGCCA ACACCTGGGC ACCTTTCCTC TTTAGTGGTC CATCGCCAGT
151 TATCACATCT GTATGCGGAA CCTCAAAGA GTCCCTGGTG TGAAGCAAGA
201 TCGCTAGAAC ACACCTTACC TGTAACAGA GAGACACTGA AAAGGAAGGT
251 TAGTGGGAAC CGTTGCGCCA GCCCTGTTAC TGGTCCAGGT TCAAAGAGGG
301 ATGCTCACTT CTGCGCTGTC TGCAGCGATT ACGCATCGGG ATATCACTAT
351 GGAGTCTGGT CGTGTGAAGG ATGTAAGGCC TTTTTTAAAA GAAGCATTCA
401 AGGACATAAT GATTATATTT GTCCAGCTAC AAATCAGTGT ACAATCGATA
451 AAAACCGGCG CAAGAGCTGC CAGGCCTGCC GACTTCGGAA GTGTTACGAA
501 GTGGGAATGG TGAAGTGTGG CTCCCGGAGA GAGAGATGTG GGTACCGCCT
551 TGTGCGGAGA CAGAGAAGTG CCGACGAGCA GCTGCACTGT GCCGGCAAGG
601 CCAAGAGAAG TGGCGGCCAC GCGCCCCGAG TCGGGGAGCT GCTGCTGGAC
651 GCCCTGAGCC CCGAGCAGCT AGTGCTCACC CTCCTGGAGG CTGAGCCGCC
701 CCATGTGCTG ATCAGCCGCC CCAGTGCGCC CTTCCCGAG GCCTCCATGA
751 TGATGTCCCT GACCAAGTTG GCCGACAAGG AGTTGGTACA CATGATCAGC
801 TGGGCCAAGA AGATTCCCGG CTTTGTGGAG CTCAGCCTGT TCGACCAAGT
851 GCGGCTCTTG GAGAGCTGTT GGATGGAGGT GTTAATGATG GGGCTGATGT
901 GCGGCTCAAT TGACCACCCC GGCAAGCTCA TCTTTGCTCC AGATCTTGTT
951 CTGGACAGGG ATGAGGGGAA ATGCGTAGAA GGAATTCCTG AAATCTTTGA
1001 CATGCTCCTG GCAACTACTT CAAGGTTTCG AGAGTTAAAA CTCCAACACA
1051 AAGAATATCT CTGTGTCAAG GCCATGATCC TGCTCAATTC CAGTATGTAC
1101 CCTCTGGTCA CAGCGACCCA GGATGCTGAC AGCAGCCGGA AGCTGGCTCA
1151 CTTGCTGAAC GCCGTGACCG ATGCTTTGGT TTGGGTGATT GCCAAGAGCG
1201 GCATCTCCTC CCAGCAGCAA TCCATGCGCC TGGCTAACCT CCTGATGCTC
1251 CTGTCCCACG TCAGGCATGC GAGTAACAAG GGCATGGAAC ATCTGCTCAA
1301 CATGAAGTGC AAAAATGTGG TCCAGTGTA TGACCTGCTG CTGGAGATGC
1351 TGAATGCCCA CGTGCTTCGC GGGTGCAAGT CCTCCATCAC GGGGTCCGAG
1401 TGCAGCCCGG CAGAGGACAG TAAAAGCAA GAGGGCTCCC AGAACCTACA
1451 GTCTCAGTGA

FIG. 13B

ATGGCATCTTAC AGTCCTGCTGTG ATGAACTACAGT GTTCCCAGCAGC ACCGGTAACCTG GAAGGTGGGCCT 72
 GTTCGCCAGACT GCAAGCCCCAAT GTGCTATGGCCA ACTTCTGACAC CTCCTCCTTTA GCCACCCACTGC 144
 CAATCATCGCTT CTCTATGCAGAA CCTCAAAAGAGT CCTTGGTGTGAA GCAAGATCACTA GAACACACCTTG 216
 CCTGTAACAGA GAGACCCTGAAG AGGAAGCTTGGC GGGAGCGTGTG GCCAGCCCTGTT ACTAGTCCAAGC 288
 ACCAAGAGGGAT GCTCACTTCTGT GCCGTCTGCAGT GATTATGCATCT GGTATCATTTAC GGTGCTGTGTC 360
 TGTGAAGGATGT AAGGCCCTTTTTT AAAAGAAGCATT CAAGGACATAAT GACTATATCTGT CCAGCCACGAA 432
 CAGTGTACGATA GACAAGAACCGG GATAAATACTGC CAGGCCCTGCCA CTTCCGCAAGTGT TACGAAGTAGGA 504
 ATGGTCAAGTGT GGATCCAGGAGA GAAAGGTGTGGG TACCCGAATAGTA CGAAGACAGAGA AGTGCCAGCGAG 576
 CAGGTGCATTGC CTGAACAAGCC AAGAGAACCAGT GGGCACACACCC CGGGTGAAGGAG CTAAGTGTGAA 648
 TCTCTGAGTCCC GAGCAGCTGGTG CTCACCCCTGCTG GAAGCTGAGCCA CCCAATGTGCTA GTGAGTCTGTC 720
 AGCATGCCCTTC ACCGAGGCCTCC ATGATGATGTCC ATGACGAAGCTG CTTGACAAGGAA CTGGTGCACATG 792
 ATTGGCTGGCC AAGAAAATCCCCT GGCTTTGTGGAG CTCAGCCCTGTTG GACCAAGTCCGC CTCATTGGA 864
 TGCTGGATGGAG GTGCTGATGGTG GGGCTGATGTGG CGTCCATCGAC CACCCCGGCAAG CTCATCTTTGCT 936
 CCAGACCTCGLT CTGGACAGGGAT GAGGGGAAGTGC GTGGAAGGATt CTGGAAATCTTT GbCATGCTCCTG 1008
 GCgACGACGGCA CGGTTCCGTGAG TTAAAACTGCAG CACAAGAATAT CTGTGTGTGAAG GCCATGATTTCTC 1080
 CTCAACTCCAGT ATGTACCACCTTG GCTACCGCCAAGC CAGGAAGCAGAG AGTAGCCGGAAG CTGACACACCTA 1152
 TTGAACGCAGTG ACAGATGCCCTG GTCTGGGTGATT TCGAAGAGTAGA ATCTCTTCCAG ATCAGTCAgTC 1224
 CGTCTGGCCAAC CTCCTGATGCTt CTCCTGATGCTt AGGCACATCAGT AACbAGGGCATG GAACATCTGCTC 1296
 AGCATGAAGTGC AAAAATGTGGTC CCGGTGTACGAC CTGCTGCTGGAG ATGCTGAATGCT CACACGCTTCCA 1368
 GGGTACAAGTCC TCAATCTCGGGg TCTGGGTGCTGC TCGACAGAGGAC AGTAAGAGCAAA GAGGGCTCCCAG 1440
 AACCTCCAGTCT CAGTGA 1458

FIG. 14B

CPD	logIC50		log IC50		IC50 (nM)		Ki (nM)		Ki (nM)		RBA(%, Ki)		RBA(%, IC50)	
	hER α	rER β	hER α	rER β	hER α	rER β	hER α	rER β	hER α	rER β	hER α	rER β	hER α	rER β
DiHydroapoandrstedione	-6.31	-6.73	485.29	187.11	245.31	163.33	0.027	0.115	0.027	0.115	0.027	0.115	0.027	0.115
Testosterone	-5.00	-5.66	10000.00	2187.76	5750.97	1937.70	0.001	0.010	0.001	0.010	0.001	0.010	0.001	0.010
DiHydrotestosterone	-6.36	-7.08	436.52	83.95	220.66	73.28	0.030	0.256	0.030	0.256	0.030	0.256	0.030	0.256
4-OH-Estradiol	-8.78	-8.67	1.66	2.14	0.95	1.89	6.934	9.892	6.934	9.892	7.889	10.037	7.889	10.037
19-Nor testosterone	-5.82	-7.22	1513.56	60.12	765.10	52.48	0.009	0.357	0.009	0.357	0.009	0.357	0.009	0.357
5 β -Androstenedione	>-4	>-4	>100000	>100000	>100000	>100000	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%
Cyproteroneacetate	>-4	>-4	>100000	>100000	>100000	>100000	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%
δ -4 androstene 3,17, dione	>-4	>-4	>100000	>100000	>100000	>100000	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%
Progesterone	>-4	>-4	>100000	>100000	>100000	>100000	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%
Corticosterone	>-4	>-4	>100000	>100000	>100000	>100000	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%
Genistein	-8.35	-9.41	4.47	0.39	2.57	0.34	2.576	54.361	2.576	54.361	2.931	55.157	2.931	55.157
β -sitosterol	>-4	>-4	>100000	>100000	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%
norethynodrel	-7.53	-7.20	29.51	63.10	14.22	53.09	0.466	0.353	0.466	0.353	0.444	0.340	0.444	0.340
norethindrone	-6.50	-5.89	316.23	1288.25	152.32	1083.89	0.043	0.017	0.043	0.017	0.041	0.017	0.041	0.017
β -zearalanol	-8.89	-9.00	1.29	0.99	0.78	0.87	8.457	21.465	8.457	21.465	10.162	21.657	10.162	21.657
D-4 androsten 3 β ,17 β -diol	-7.33	-7.64	46.77	22.91	23.39	18.71	0.283	1.001	0.283	1.001	0.280	0.937	0.280	0.937
dieneestrol	-10.03	-10.46	0.09	0.03	0.05	0.03	140.523	661.418	140.523	661.418	138.995	618.871	138.995	618.871
Methoxychlor	-5.45	-6.96	3548.13	109.65	1774.07	89.56	0.004	0.209	0.004	0.209	0.004	0.196	0.004	0.196
Bisphenol A	-6.41	-7.37	389.05	42.66	194.52	34.84	0.034	0.538	0.034	0.538	0.034	0.503	0.034	0.503
Ecdysterone*	>-4	>-4	>100000	>100000	>100000	>100000	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%
Eudesmine	>-4	>-4	>100000	>100000	>100000	>100000	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%
Lapidine	>-4	>-4	>100000	>100000	>100000	>100000	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%	<0.0002%
Tschimgine	-7.18	-6.63	66.07	234.42	40.13	206.47	0.165	0.091	0.165	0.091	0.198	0.092	0.198	0.092
Tschimganidine	-7.87	-6.45	13.49	354.81	8.19	312.50	0.808	0.060	0.808	0.060	0.971	0.060	0.971	0.060
Ferutinine	-9.10	-9.56	0.79	0.27	0.40	0.24	16.623	78.091	16.623	78.091	16.623	78.091	16.623	78.091
Coumestrol	-9.65	-10.12	0.22	0.08	0.14	0.07	48.665	282.307	48.665	282.307	58.479	284.839	58.479	284.839
Nafoxidine	-9.32	-9.05	0.48	0.90	0.24	0.78	27.530	23.966	27.530	23.966	27.530	23.966	27.530	23.966
16a-Br-E2	-9.88	-9.67	0.13	0.21	0.07	0.19	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000
17a-E2	-9.44	-8.88	0.36	1.32	0.22	1.16	30.006	16.133	30.006	16.133	36.058	16.278	36.058	16.278
17 β -E2	-9.68	-9.87	0.21	0.13	0.13	0.12	52.145	157.660	52.145	157.660	62.661	159.074	62.661	159.074
*1 β -Ecdysone, 20_Hydroxyecdysone														
RBA-values derived from 16a-Br-E2 (100%)														

FIG. 15

ORPHAN RECEPTOR

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a Continuation of U.S. Ser. No. 10/083,807 filed Feb. 27, 2002, now abandoned, which is a Continuation of U.S. Ser. No. 09/333,057 filed Jun. 14, 1999, now abandoned, which is a Continuation of U.S. Ser. No. 08/836,620, filed May 8, 1997, now U.S. Pat. No. 5,958,710, which is a 371 and claims the benefit of International Application Serial No. PCT/EP96/03933 filed Sep. 9, 1996. All the above applications are incorporated by reference in their entireties.

This invention relates to cellular nuclear receptors and their uses.

A large family of nuclear receptors which confer cells with responsiveness to molecules such as retinoid acid, vitamin D, steroid hormones and thyroid hormones has been identified. Extensive studies have shown that the members of this superfamily of nuclear receptors activate and/or repress gene transcription through direct binding to discrete cis-acting elements termed “hormone response elements” (HRE). It has been shown that these HRE’s comprise repeats of consensus palindromic hexanucleotide DNA motifs. The specificity of the HRE’s is determined by the orientation of, and spacing between, halvesites (i.e. half a palindromic sequence)(Umenesono K., et al, 1991 *Cell* 65, 1255–1266).

Specific DNA binding is mediated by a strongly-conserved DNA binding domain, containing two zinc fingers, which is conserved among all thus discovered nuclear receptors. Three amino acids at the C-terminal base of the first zinc finger (known as the “P-box”) are important for the recognition of the half site nucleotide sequence. Members of the nuclear receptor superfamily have been classified into different groups on the basis of the amino acid sequence within the P box.

All members of the nuclear receptor superfamily also contain a hypervariable N-terminal domain and a ligand-binding domain containing some “patches” of conserved sequence. One of these is called the “Ti-domain”.

Molecules which are thought to be nuclear receptors, as they are structurally related to characterised receptors, but for which no ligand has been found, are termed “orphan receptors”. Many such orphan receptors have been identified (see for example Evans R. M. (1988) *Science* 240, 889–895 and O’Malley, B. (1990) *Mol. Endocrinol.* 4 363–369)

We have now unexpectedly identified, initially in rat a new orphan receptor, which is related to the known estrogen receptor ER α , and which we have designated “ER β ” (specifically “rER β ” in rat). In this specification “Er β ” will be used to refer to the receptors hER β or rER β or related receptors. The nucleotide and amino acid sequences of rER β have now been determined and are shown in FIG. 1. We have also identified a human Er β —“hER β ”, the amino acid DNA and sequences of which are shown in FIGS. 13A and 13B respectively.

According to one aspect of the invention there is provided a novel estrogen receptor-related nuclear receptor, herein-after termed “ER β ” having the amino acid sequence of FIG. 1, FIG. 13A or 16A or substantially the same amino acid sequence as the amino acid sequence shown in FIG. 1, 13A or 16A or an amino acid sequence functionally similar to those sequence. The isolated receptor may be particularly useful in the search for molecules for use in treatment of diseases or conditions such as cardiovascular diseases, cen-

tral nervous system diseases or conditions or osteoporosis, prostate cancer or benign prostatic hyperplasia.

The receptor of the invention may also be used in the testing of environmental chemicals for estrogenic activity.

5 There has been increasing concern over the effect of various chemicals released into the environment on the reproduction of humans and animals. Threats to the reproductive capabilities of birds, fish, reptiles, and some mammals have become evident and similar effects in humans have been proposed. Substantial evidence is now emerging which shows that exposure to certain chemicals during critical periods of foetal life may distort the development of the reproductive organs and the immune and nervous systems. 10 On the basis of possible parallels between actual wildlife effects, seen for example in birds and seals living in highly polluted areas, and proposed effects in humans, in combination with documented human reproductive effects caused by prenatal exposure to the pharmaceutical estrogen, diethyl stilbestrol (DES), “estrogenic” chemicals have been proposed to threaten the reproductive capability of both animals and humans. Among the chemicals known or suspected to act as estrogen mimics on the human body, or in other ways disturb the human endocrine system, there are several which 15 have already been identified as environmental hazards. Among the chemicals that have been mentioned as potential causes of disruption of reproductive function in animals and humans are chlorinated organic compounds such as dieldrin, endosulfans, chlordanes, endrins, aldrin, DDT and some 20 PCBs, plastics such as Bisphenol A, phthalates and non-ylphenol, and aromatic hydrocarbons. Some of the proposed effects on humans have been suggested to be due to an increasing exposure to environmental estrogens—in fact, exposure to chemical compounds to which higher organisms during the foetal period react in a way that is similar to when they are exposed to high dosages of estrogens. The effects are manifested by for example perturbations of the sex characteristics and impaired reproductive potential. In humans, elevated risks of breast cancer and other hormone-related disease has also been discussed as possible effects. In addition, to the documented “estrogenic” effects, it has recently been demonstrated that environmental pollutants may also act on hormonal pathways other than the estrogenic pathway—it has been shown that p,p’—DDE the main 25 metabolite of DDT (also in humans) is a fairly anti-androgenic agent (Kelce W. R. et al *Nature* 1995 375:581–585). Epidemiological studies on these issues are, however, presently difficult to interpret. Nevertheless, there is a growing opinion against these potentially hormone disrupting chemicals, and very palpable public and environmental demand for the governmental agencies and industry to act. In view of the similarities between the receptor of the present invention, Er β and the classical estrogen receptor, Er β may be used in the testing of chemicals for estrogenic effect. 30

35 An amino acid sequence functionally-similar to the sequence shown in FIG. 1, 13A or 14A may be from a different mammalian species.

40 An amino acid sequence which is more than about 89%, identical with the sequence shown in FIG. 1, 13A or 14A is substantially the same amino acid sequence for the purposes of the present application. Preferably, the amino acid sequences is more than about 95% identical with the sequence shown in FIG. 1, 13A or 14A.

45 According to another aspect of the invention there is provided a DNA sequence encoding a nuclear receptor according to the first aspect of the invention. Preferably, the

DNA sequence is that given in FIG. 1, 13A or 14A or is a DNA sequence encoding a protein or polypeptide having the functionality of ER β .

ER β is unique in that it is extremely homologous to the rat estrogen receptor, in particular in its DNA binding domain. It appears that ER β has a very limited tissue distribution. In female rats, it appears to be present only in the ovaries, and in male rats in the prostate and testes. As these tissues are classic targets for estrogen action, it can be deduced that ER β may mediate some of the effects of estrogen.

The different ligand specificity of ER α and ER β may be exploited to design pharmaceutical agents which are selective for either receptor. In particular, the differences in ligand specificity may be used to develop drugs that specifically target cardiovascular disease in postmenopausal women or osteoporosis.

The nuclear receptor of the invention, ER β , a method of producing it, and tests on its functionality will now be described, by way of example only, with reference to the accompanying drawings, FIGS. 1 to 15 in which:

FIG. 1 shows the amino acid sequence of ER β and the nucleotide sequence of the gene encoding it;

FIG. 2A is a phylogenetic tree showing the evolution of ER β and other receptors;

FIG. 2B shows the homology between the different domains in ER β and certain other receptors;

FIG. 2C is an alignment of the amino acid sequence in the ligand binding domains of rER β , rER α , mER α and hER α ;

FIG. 2D is an alignment of the amino acid sequence in the DNA binding domains of rER β , rER α , mER α and hER α ;

FIG. 3A is a film autoradiograph of prostate gland showing strong expression of a clone of the receptor of the invention, clone 29;

FIG. 3B is a darkfield image showing prominent signal for clone 29 in epithelium (e) of prostatic alveoli. The stroma(s) exhibit(s) weaker signal;

FIG. 3C is a bipolarization image of cresyl violet counterstained section showing silver grains over epithelium (e), whereas the stroma(s) contain(s) less grains;

The bar represents 0.7 mm for FIG. 3A, 200 μ m for FIG. 3B and 30 μ m for FIG. 3C;

FIG. 4A shows a film autoradiograph of ovary showing strong expression of clone 29 in follicles at different developmental stages (some are indicated by arrows). The interstitial tissue (arrowheads) shows low signal;

FIG. 4B shows a darkfield image showing high expression of clone 29 in granular cells of primary (1), secondary (2), tertiary (3) and mature (4) follicles. Low signal is present in interstitial tissue (it);

FIG. 4C is a bipolarization image of ovary showing strong signal in granular cells (gc), whereas the oocyte (oc) and the cainterna (ti) are devoid of clear signal;

The bar represents 0.9 mm for FIG. 4A, 140 μ m for FIG. 4B and 50 μ m for FIG. 4C;

FIG. 5A illustrates the results of saturation ligand binding analysis of cloned ER β ;

FIG. 5B illustrates the specificity of ligand binding by cloned ER β ;

FIG. 5C illustrates E2 binding by ER β ;

FIG. 6 illustrates the activation of transcription by cloned ER β ;

FIGS. 7 and 7A illustrates stimulation by various ligands by cloned ER β ;

FIG. 8 illustrates the results of RT-PCR experiments on the expression of rat estrogen receptors;

FIG. 9 illustrates the results of RT-PCR experiments on the expression of human Er β (hER β);

FIG. 10A is a Hill plot comparing binding of 125 I-E2 by hER α and rER β ;

FIG. 10B is a Scatchard plot comparing binding of 125 I-E2 by hER α and rER β ;

FIG. 11A illustrates the relative binding affinity of hER α and rER β for various ligands;

FIG. 11B is a detail of FIG. 12A;

FIG. 12 is an alignment of various estrogen receptors;

FIG. 13A shows the amino acid sequence of human ER β ;

FIG. 13B shows the DNA sequence of human Er β ;

FIG. 14A shows the amino acid sequence of mER β ;

FIG. 14B shows the DNA sequence of mouse Er β ; and

FIG. 15 illustrates ligand binding affinities for various phytoestrogens by ER's of the invention.

A. CLONING OF RAT ER β

1. PCR-Amplification and Complementary DNA Cloning.

A set of degenerate primers (DBD 1, 2, 3 and WAK/FAK) were designed previously according to the most highly conserved sequences of the DNA-binding domain (P-box) and ligand binding domain (Ti-stretch) of members of the nuclear receptor family (Enmark, E., Kainu, T., Pelto-Huikko, M., & Gustafsson, J-Å (1994) *Biochem. Biophys. Res. Commun.* 204, 49–56). Single strand complementary DNA reverse transcribed from rat prostate total RNA was employed with the primers in PCR reactions as described in Enmark, E., Kainu, T., Pelto-Huikko, M., & Gustafsson, J-Å (1994) *Biochem. Biophys. Res. Commun.* 204, 49–56. The amplification products were separated on a 2% low melting agarose gel and DNA products between 400 and 700 bp were isolated from the gel and ligated to TA cloning vector (Invitrogen). As alternatives, we also used the RP-I/IRP-2 and DBD66–100/DBD210–238 primer sets in the DNA-binding domain of nuclear receptors exactly as described by Hirose T., Fijimoto, W., Yamaai, T., Kim, K. H., Matsuura, H., & Jetten, A. M. (1994) *Mol. Endocrinol.* 8, 1667–1677 and Chang, C., Lopes Da Silva, S., Ideta, R., Lee, Y., Yeh, S., & Burbach, J. P. H. (1994) *Proc. Natl. Acad. Sci.* 91, 6040–6044 respectively. Clone number 29 (obtained with the DBD-WAK/FAK set) with a length of 462 bp showed high homology (65%) with the rat estrogen receptor cDNA (65%), which was previously cloned from rat uterus (Koike, S., Sakai, M., & Muramatsu, M. (1987) *Nucleic Acids Res* 15, 2499–2513). The amino acid residues predicted by clone 29 DNA sequences suggested that this DNA fragment encoded part of the DNA-binding domain, hinge region and the beginning of the ligand binding domain of a novel member of the nuclear receptor family. Two PCR primers (FIG. 1) were used to generate a probe of 204 bp consisting of the hinge region of the novel receptor, which was used to screen a rat prostate cDNA library (Clontech gt10) under stringent conditions resulting in four strongly positive clones with a size of 0.9 kb, 1.8 kb, 2.5 kb and 5–6 kb respectively. The clone of 2.5 kb was sequenced and FIG. 1 shows the nucleotide sequence determined in the core facility (CyberGene AB) by cycle sequencing using fluorescent terminators (Applied Biosystems) on both strands, with a series of internal primers and deduced amino acid sequence of clone 29. Two in frame ATG codons are located at nucleotide 424 and nucleotide 448, preceding by an in-frame stop codon at nucleotide 319, which suggests that they are possible start codons. The open reading frame encodes a protein of 485 amino acid residues (counted from the first methionine) with a calculated molecular weight of 54.2 kDa. Analysis of the proteins by synthesized by in-vitro transla-

tion from the clone 29 cRNA in rabbit reticulocyte lysate revealed a doublet protein band migrating at approximately 57 kDa on SDS-PAGE gels (data not shown), confirming the open reading frame. The doublet protein band is probably caused by the use of both ATG codons for initiation of protein synthesis. The amino acid sequence of clone 29 protein shows the characteristic zinc module DNA-binding domain, hinge region and a putative ligand binding domain, which are the characteristic features of members of the nuclear receptor family (Tsai, M.-J., & O'Malley, B. W. (1994) *Ann. Rev. Biochem.* 63, 451–486; Härd, T., & Gustafsson, J.-Å (1993) *Acc. Chem. Res.* 26, 644–650; Laudet, V., Hänni, C., Coli, J., Catzeflis, F., & Stehelin, D (1992) *EMBL J* 11, 1003–1012).

Protein sequence comparison with several representative members of the nuclear receptor family (FIG. 2) showed the clone 29 protein is most related to the rat estrogen receptor (ER α), cloned from uterus (Koike, S., Sakai, M., & Muramatsu, M. (1987) *Nucleic Acids Res.* 15, 2499–2513), with 95% identity in the DNA-binding domain (amino acid residues 103–167) (Griffiths, K., Davies, P., Eaton, C. I., Harper, M. E., Turkes, A., & Peeling, W. B. (1991) in *Endocrine Dependent Tumours*, eds. Voigt, K.-D. & Knabbe, C. (Raven Press), pp. 83–125). A number of functional characteristics have been identified within the DNA-binding domain of nuclear receptors (Härd, T., & Gustafsson, J.-Å. (1993) *Acc. Chem. Res.* 26, 644–650 and Zilliaccus, J., Carlstedt-Duke, J., Gustafsson, J.-Å., & Wright, A. P. H. (1994) *Proc. Natl. Acad. Sci. USA* 91, 4175–4179). The so-called P-box specifies nucleotide sequence recognition of the core half-site within the response element, while the D-box mediates dimerization between receptor monomers. The clone 29 protein P-box and D-box sequences of EGCKA and PATNQ, respectively, are identical to the corresponding boxes in ER α (Härd, T., & Gustafsson, J.-Å. (1993) *Acc. Chem. Res.* 26, 644–650 and Koike, S., Sakai, M., & Muramatsu, M. (1987) *Nucleic Acids Res.* 15, 2499–2513), thus predicting that clone 29 protein binds to ERE sequences.

The putative ligand binding domain (LBD) of clone 29 protein (amino acid residues 259–457) shows closest homology to the LBD of the rat ER α (FIG. 2), while the homology with the human ERR1 and ERR2 proteins (Giguere, V., Yang, N., Segui, P., & Evans R. M. (1988) *Nature* 331, 91–94) is considerably less. With the human, mouse and xenopus estrogen receptors the homology in the LBD is also around 55%, while the homology with the LBD of other steroid receptors is not significant (FIG. 2). Cysteine residue 530 in human ER α has been identified as the covalent attachment site of an estrogenic affinity label (Harlow, K. W., Smith D. N., Katzenellenbogen, J. A., Greene, G. L., & Katzenellenbogen, B. S. (1989) *J. Biol. Chem.* 264, 17476–17485). Interestingly, clone 29 protein (Cys-436) as well as the mouse, rat and xenopus ER α s have a cysteine residue at the corresponding position. Also, two other amino acid residues described to be close to or part of the ligand-binding pocket of the human ER α -LBD (Asp 426 and Gly 521) are conserved in the LBD of clone 29 protein, (Asp 333 and Gly 427) and in the LBD of ER α s from various species (20,21). The ligand-dependent transactivation function TAF-2 identified in ER α (Danielian, P. S., White, R., Lees, J. A., & Parker, M. G. (1992) *EMBO J.* 11, 1025–1033), which is believed to be involved in contacting other transcription factors and thereby influencing activation of transcription of target genes, is almost completely conserved in clone 29 protein (amino acid residues 441–457). Steroid hormone receptors are phosphoproteins (Kuiper, G., & Brinkmann, A. O. (1994) *Mol. Cell. Endocrinol.* 100, 103–107), and several phosphorylation sites identified in the

N-terminal domain and LBD of ER α (Arnold, S. F., Oboum, J. D., Jaffe, H., & Notides, A. C. (1995) *Mol. Endocrinol.* 9, 24–33 and Le Goff, P., Montano, M. M., Schodin, D. J., & Katzenellenbogen, B. S. (1994) *J. Biol. Chem.* 269, 4458–4466) are conserved in clone 29 protein (Ser 30 and 42, Tyr 443). Clone 29 protein consists of 485 amino acid residues while ER α s from human, mouse and rat consist of 590–600 amino acid residues. The main difference is a much shorter N-terminal domain in clone 29 protein i.e. 103 amino acid residues as compared to 185–190 amino acid residues in the other receptor proteins. Also the non-conserved so-called F-domain at the C-terminal end of ER α s is 15 amino acid residues shorter in clone 29 protein. The cDNA insert of a positive clone of 2.6 kb was subcloned into the EcoRI site of pBluescript (trademark) (Stratagene). The complete DNA sequence of clone 29 was determined (CyberGene AB) by cycle sequencing using fluorescent terminators (Applied Biosystems) on both strands, with a series of internal primers.

FIGS. 2C and 2D respectively compare the ligand and DNA binding domain of Er β compared to rat, mouse and human Er α 's .

2. Saturation Ligand Binding Analysis and Ligand Competition Studies:

Clone 29 cDNA was subcloned in pBluescript downstream of the T7 promoter to give p29-T7. Clone 29 protein was synthesized in vitro using the TnT-coupled reticulocyte lysate system (Promega). Translation reaction mixtures were diluted five times with TEDGMO buffer (40 mM Tris/HCl, pH 7.4, 1mM EDTA, 10% (v/v) glycerol, 10 mM Na₂MoO₄, 10 mM DTT) and 0.1 ml aliquots were incubated for 16 h at 8° C. with 0.3–6.2 nM [2,4,6,7-³H]-17 β -estradiol (NEN-Dupont; specific radioactivity 85 Ci/mmol) in the presence or absence of a 200-fold excess of unlabelled E2.

FIG. 5A illustrates the results of a saturation ligand analysis of clone 29 protein. Reticulocyte lysate containing clone 29 protein was incubated with 6 concentrations of [³H]E2 between 0.3 and 6.0 nM. Parallel tubes contained an additional 200 fold of non-radioactive E2. Bound and free ligand were separated with a dextran-coated charcoal assay. The K_d (0.6 nM) was calculated from the slope of the line in the Scatchard plot shown (r=0.93), and the number of binding sites was extrapolated from the intercept on the abscissa (B_{max}=1400 fmol/ml undiluted translation mixture).

For ligand competition studies diluted reticulocyte lysate was incubated with 5 nM [2,4,6,7-³H]-17 β -estradiol in the presence of either 0, 5, 50, 500 or 5,000 nM of non-radioactive E2, estrone, estriol, testosterone, progesterone, corticosterone, 5 α -androstane-3 β ,17 β -diol, 5 α -androstane-3 α ,17 β -diol and diethylstilbestrol (DCES) for 16 h at 8° C. Bound and unbound steroids were separated with a dextran-coated charcoal assay (Ekman, P., Barrack, E. R., Greene, G. L., Jensen, E. V., & Walsh, P. C. (1983) *J. Clin. Endocrinol Metab.* 57, 166–176).

FIG. 5B illustrates the specificity of ligand binding by clone 29 protein. Reticulocyte lysate containing clone 29 protein was equilibrated for 16 h with 5 nM [³H]E2 and the indicated fold excess of competitors. Data represent [³H]E2 bound in the presence of unlabelled E2, testosterone (T), progesterone (prog), corticosterone (cortico), estrone (E1), diethylstilbestrol (DES), 5 α -androstane-3 α , 17 β -diol (3 α -AD), 5 α -androstane-3 β , 17 β -diol (3 β -AD) and estriol (E3). [³H]E2 binding in the absence of competitor was set at 100%.

3. In-Situ Hybridisation:

In-situ hybridisation was carried out as previously described (Dagerlind Å., Friberg, K., Bean, A. J., & Hökfelt, T (1992) *Histochemistry* 98, 39–49). Briefly, two oligo-

nucleotide probes directed against nucleotides 994–1041 and 1981–2031 were each labelled at the 3'-end with ^{33}P -dATP using terminal deoxynucleotidyltransferase (Amersham, UK). Adult male and female Sprague-Dawley rats (age 2 to 3 months $n=10$) were used for this study. The rats were decapitated and the tissues were rapidly excised and frozen on dry ice. The tissues were sectioned in a Microm HM500 cryostat at $14\ \mu\text{m}$ and thawed onto Probe-On glass slides (Fisher Scientific, PA, USA). The slides were stored at -20°C . until used. The slides were incubated in humidified boxes at 42°C . for 18 h with 1×10^6 cpm of the probe in a hybridization solution containing 50% formamide, $4\times\text{SSC}$ ($1\times\text{SSC}=0.15\ \text{M NaCl}$, $0.015\ \text{M sodium citrate}$), $1\times$ Denhardt (0.02% BSA, 0.02% Ficoll, 0.02% PVP), 1% sarkosyl, 0.02 M sodium phosphate (pH 7.), 10% dextran-sulphate, 500 $\mu\text{g/ml}$ salmon sperm DNA and 200 mM DTT. Slides were subsequently rinsed in $1\times\text{SSC}$ at 55°C . for 60 min with four changes of SSC and finally in $1\times\text{SSC}$ starting at 55°C . and slowly cooled to room temperature, transferred through distilled water and briefly dehydrated in 50% and 95% ethanol for 30 sec each, air-dried, and covered with Amersham β -man autoradiography film for 15 to 30 days. Alternatively the slides were dipped in Kodak NTB2 nuclear track emulsion (diluted 1:1 with distilled water) and exposed for 30 to 60 days at 4°C . Finally, the sections were stained with cresyl violet.

Clear expression of clone 29 was observed in the reproductive tract of both male and female rats, while in all other rat tissues the expression was very low or below the level of detection with in-situ hybridisation (not shown). In male reproductive organs high expression was seen in the prostate gland (FIG. 3), while very low expression was observed in testis, epididymis and vesicula seminalis (not shown). In dipped sections, expression was clearly visible in prostate epithelial cells (secreting alveoli) while the expression in smooth muscle cells and fibroblasts in the stroma was low (FIG. 3). In female reproductive organs expression was seen in the ovary (FIG. 4), while uterus and vagina were negative (not shown). In dipped sections high expression was seen in the granulosa cell layer of primary, secondary and mature follicles (FIG. 4), whereas primordial follicles, oocytes and corpora lutea appeared completely negative. Low expression was seen in the interstitial cells of the ovary. Both anti-sense oligonucleotide probes used produced similar results. Addition of a 100 fold excess of the respective unlabelled oligonucleotide probes during the hybridisation reactions abolished all signals.

4. Transactivation Analysis in CHO-Cells:

The expression vector pCMV29 was constructed by inserting the 2.6 kb clone 29 fragment in the EcoRI site of the expression vector pCMV5 (Andersson, S., Davis, D.L., Dahlbäck, H., Jörnvall, H., & Russell, D.W. (1989) *J. Biol. Chem.* 264, 8222–8229). The pERE-ALP reporter construct contains a secreted form of the placental alkaline phosphatase gene (Berger, J., Hauber, J., Hauber, R., Geiger, R., & Cullen, B.R. (1988) *Gene* 66 1–10) and the MMTV-LTR in which the glucocorticoid response elements were replaced by the vitellogenin promoter estrogen response element (ERE). The vector pCMV29 containing the clone 29 fragment was deposited with NCIMB, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, Scotland, UK AB21 9YA in accordance with the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure on 26 May 2006, under accession No. NCIMB 41404. The deposit was named “*Escherichia coli* DH5 prat ER (CmV29)”. All restrictions on the availability to the public of the material deposited will be irrevocably removed upon the granting of a patent.

CHO-K1 cells were seeded in 12-well plates at approximately 1.7×10^5 cells per well in phenol-red free Ham F12

medium with 5% FCS (dextran-coated charcoal treated) and 2 mM Lglutamine. After 24 h the cells were transfected with 250 ng pERE-ALP vector and 50 ng pCMV29 using lipofectamine (Gibco) according to the manufacturer's instructions. After five hours of incubation the cells were washed and refed with 0.5 ml phenol-red free Coon's F-12 medium containing 5% serum substitute (SRC 3000, Tissue Culture Services Ltd., Botolph Claydon, Buckingham, UK) 2 mM Lglutamine and 50 $\mu\text{g/ml}$ gentamicin plus hormones as indicated. After 48 h the medium was assayed for alkaline phosphatase (ALP) activity by a chemiluminescence assay. A 10 μl aliquot of the cell culture medium was mixed with 200 μl assay buffer (10 mM diethanolamine pH 10.0 1 mM MgCl_2 and 0.5 mM CSPD (Tropix Inc. Boston, USA)) and incubated for 20 min at 37°C . before measurement in a microplate luminometer (Luminoskan; Labsystems, Finland) with integral measurement for 1 second. The ALP activity of ERE-reporter alone was set at 1.

5. Ligand Binding Characteristics and Transactivation Function of Clone 29 Protein:

On the basis of the described high homology between clone 29 protein and rat ER α in the DBD and LBD it was hypothesized that clone 29 protein might encode a novel ER. Furthermore, biological effects of estrogens on rat prostate and ovary, which show high expression of clone 29 RNA, are well known (Griffiths, K., Davies, P., Eaton, C. I., Harper, M. E., Turkes, A., & Peeling W. B. (1991) in *Endocrine Dependent Tumours*, eds Voigt, K-D. & Knabbe, C. (Raven Press), pp 83–125; Richards, J. S (1994) *Endocrine Rev.* 15, 725–745; and Habenicht, U-F., Tunn, U. W., Senge, Th., Schroder, R. H., Schweikert, H. U., Bartsch, G., & E1 Etreby, M. F. (1993) *J. Steroid Biochem. Molec. Biol.* 44, 557–563). In order to analyze the steroid binding properties of clone 29 protein synthesized in vitro, the reticulocyte lysate was incubated at 8°C . for 16 h with increasing concentrations (0.3–6.0 nM) of [^3H]E2 in the presence or absence of a 200 fold molar excess of unlabelled E2. Linear transformation of saturation data-revealed a single population of binding sites for E2 with a K_d (dissociation constant) of 0.6 nM (FIGS. 5A and C). Steroid binding specificity was measured by incubating reticulocyte lysate with 5 nM [^3H] E2 in the presence of 0.5, 50, 500 and 5,000 nM unlabelled competitors. Competition curves generated are indicative of an estrogen receptor in that only estrogens competed efficiently with [^3H]E2 for binding (FIG. 5B). Fifty percent inhibition of specific binding occurred by 0.6 fold excess of unlabelled E2; diethylstilbestrol, estriol, estrone and 5α -androstane- $3\alpha,17\beta$ -diol were 5, 15, 50 and 150 times, respectively, less effective as competitors. Neither testosterone, progesterone, corticosterone nor 5α -androstane- $3\alpha,17\beta$ -diol were efficient competitors, even at the highest concentrations used (1000 fold excess). The dissociation constant and the steroid binding specificities measured are in good agreement with data previously reported for ERs in rat and human prostate, rat granulosa cells, rat antral follicles and whole rat ovarian tissue (Ekman, P., Barrack, E. R., Greene, G. L., Jensen, E. V., & Walsh. P. C (1983) *J. Clin. Endocrinol. Metab.* 57, 166–176; van Beurden-Lamers, W. M. O., Brinkmann, A. O., Mulder, E., & van der Molen, H. (1974) *Biochem. J* 140, 495–502; Kudolo, G. B., Elder, M. G., & Myatt, L. (1984) *J. Endocrinol.* 102, 83–91; and Kawashima, M., & Greenwald, G. S. (1993) *Biology of Reprod.* 48 172–179).

When clone 29 protein was labelled with a saturating dose of [^3H]E2 and analyzed on sucrose density gradients, a single peak of specifically bound radioactivity was observed. The sedimentation coefficient of this complex was about 7S, and it shifted to 4S in the presence of 0.4 M NaCl (not shown). To investigate the transcriptional regulatory properties of clone 29 protein, we performed co-transfection

experiments in which CHO cells were transfected with a clone 29 protein expression vector and/or an estrogen-responsive reporter gene construct. Cells were incubated in the absence of E2 (clone 29) or in the presence of 100 nM E2 (Clone 29+E2) or in the presence of 100 nM E2 and 12 μ M Tamoxifen (Clone 29+E2/Tam). In the absence of exogenously added E2 clone 29 protein showed considerable transcriptional activity which could be further increased by the addition of 100 nM E2 (FIG. 6). Simultaneous addition of a 10 fold excess of the antiestrogen Tamoxifen partially suppressed the E2 stimulated activity (FIG. 6). The constitutive transcriptional activity of clone 29 protein could be suppressed by the anti-estrogen ICI-1624384 (not shown). It has been shown previously that the wild-type mouse and human ERs are constitutive activators of transcription, and that the transcriptional activity can be stimulated further by the addition of E2 (Txukernan, M., Xiao-Kun Zhang., Hermann, T., Wills, K. N., Graupner, G., & Phal, M. (1990) *New Biologist* 2, 613–620 and Lees, J. A., Fawell, S. E., & Parker, M. G. (1989) *Nucl. Acids Res.* 17, 5477–5488). To obtain more insight into what concentrations of E2 effect clone 29 protein transcriptional activity, transient transfection experiments were carried out in the presence of increasing concentrations of E2. CHO-cells were transiently transfected with the ERE-reporter plasmid and the clone 29 protein expression plasmid. Cells were incubated with increasing concentrations of E2 (0.1–1000 nM), estrone (E1, 1000 nM), 5 α -androstane-3 β ,17 β -diol (3 β -AD, 1000 nM) or no ligand added. Alkaline phosphatase activity (ALP) was measured as described and the activity in the absence of ligand (control) was set at 1. The figure shows relative ALP-activities (\pm SD) from three independent experiments. Clone 29 protein began to respond at 0.1 nM E2 and maximal stimulation was observed between 1 nM and 10 nM E2 (FIG. 7). The maximal stimulation factor was 2.6 \pm 0.5 fold (mean \pm SD, n=9) as compared to incubation in the absence of E2. Apart from E2 also estrone and 5 α -androstane-3 β , 17 β -diol could stimulate transcriptional activity, albeit at higher concentrations (FIG. 7). Dexamethasone, testosterone, progesterone, 5 α -androstane-3 α ,17 β -diol, thyroid hormone and all-trans-retinoic acid could not stimulate transcriptional activity of clone 29 protein, even at the highest concentration (1000 nM) tested (not shown). The results of the co-transfection experiments are in agreement with the ligand binding and specificity data of clone 29 protein presented in FIG. 5. In control experiments, wild-type human ER α also showed transcriptional activity in the absence of E2, which could be increased by the addition of E2 (not shown).

6. Detection of Rat ER Expression by RT-PCR

The tissue specificity of expression of rat ER β and ER α was determined using reverse transcriptase polymerase chain reaction (RT-PCR). The results of the experiment are shown in FIG. 8.

B. Isolation of Human Er β

1. A human version of Er β (hER β) has also been cloned from human ovary. The tissue specificity of hER β expression in a variety of cells was also determined using the RT-PCR technique. The results are shown in FIG. 9. It will be noticed that there is a very high level of mRNA of hER β in human umbilical vein endothelial cells (HUVEC) but no detection of hER α in the same cells. In addition, it will be seen that in human osteosarcoma cell line (HOS-D4), hER β is expressed in greater quantities compared to hER α .

I. A human version of ER β (hER β) has also been cloned. The tissue specificity of hER β expression in a variety of cells was also determined using the RT-PCR tech-

nique. The results are shown in FIG. 9. It will be noticed that there is a very high level of mRNA of hER β in human umbilical vein endothelial cells (HUVEC) but no detection of hER α in the same cells. In addition, it will be seen that in human osteosarcoma cell line (HOS-D4), hER β is expressed in greater quantities compared to hER α .

The partial DNA sequence of hER β is shown in FIG. 10 and a derived amino acid sequence is shown in FIG. 11.

10 Cloning of Human Er β from Testis

A commercially available cDNA from human testis (Clontech, article no. HL1161x) was screened, using a fragment containing the ligand-binding domain of the rat Er β cDNA as probe. Approximately 10⁶ recombinants were screened, resulting in one positive clone. Upon sequencing of this clone, it was seen that the insert was 1156 bp (FIGS. 13A and 13B). This corresponds to most of the translated region of a receptor with an overall homology of 90.0% to rat Er β , therefore deduced to represent the human form of Er β .

The cloned hER β , however, lacks approximately 47 amino acids at the N-terminal end and 61 amino acids at the C-terminal end (as compared to the rat sequence). Further screening of the same library was unsuccessful. PCR technology was therefore used to obtain the remaining parts. For oligonucleotides were synthesised; two degenerate oligonucleotides containing all possible codons for the amino acids adjacent to the initiation methionine and the stop codon, respectively, of the rat Er β , and two specific oligonucleotides containing the sequence of the clone isolated from the human testis library and situated approximately 100 bp from respective end of this clone. PCR with the N-terminal and C-terminal pair of oligos yielded specific bands, that were subcloned and sequenced. The parts of these new clones that overlap the original cDNA clone are identical to this. It was thus possible to construct peptide and DNA sequences corresponding to the whole open reading frame (FIGS. 13A and 13B).

When comparing the human Er β to rat Er β , this receptor is 79.6% identical in the N-terminal domain, 98.5% in the DNA-binding domain, 85.6% in the hinge and 91.6% in the ligand-binding and F-domains. These numbers match very well those found when comparing the rat and human forms of ER α .

Studies of the expression of human Er β using Northern blot show expression in testis and in ovaries. The expression in prostate, however, appears lower than found in the rat.

The human Er β gene has been mapped to chromosome 14 using PCR and to region 14q22–23 using the FISH technique, whereas the human Er β gene has been mapped to chromosome 6q25.

2. Comparison of ligand binding affinity of hER α and rER β

The ligand affinity of the two estrogen receptors, human ER α (ovary) (hER β) and rat Er β (rER β) was tested in binding saturation experiments and in binding competition experiments.

cDNA of the receptor subtypes hER α and rER β were in vitro translated in rabbit reticulocyte lysate in presence of non-radioactive amino acids according to the instructions supplied by the manufacturer (Promega).

The radioactive ligand used in all experiments was 16 α [¹²⁵I]-17 β -estradiol ([¹²⁵I]-E2) (NEX-144, New England Nuclear). The method for the binding experiments was previously described in: Salomonsson M, Carlsson B, Haggblad J. J. *Steroid Biochem. Molec. Biol.* Vol. 50, No. 5/6 pp. 313–18, 1994. In brief, estrogen receptors are incubated with [¹²⁵I]-E2 to equilibrium (16–18 h at +4° C.). The incubation was stopped by separation of protein-bound

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[¹²⁵I]-E2 from free [¹²⁵I]-E2 on Sephadex G25 columns. The radioactivity of the eluate is measured in a gamma-counter.

In the competition experiments, non-radioactive ligands were diluted in DMSO, mixed with [¹²⁵I]-E2 (approximately 100–200 pM), aliquoted in parallel, and finally hER α or rER β was added. The final concentration of DMSO in the binding buffer was 2%.

The buffer used in the experiments was of the following composition: Hepes (pH=7.5) 20 mM, KCl 150 mM, EDTA 1 mM, glycerol (8.7%), monothioglycerol 6 mM, Na₃MO₄ 10 mM.

3. Equilibrium binding saturation experiments (K_d -determinations)

A range of concentrations of [¹²⁵I]-E2 were mixed with the ER:s and incubated as described above, free [¹²⁵I]-E2 was determined by subtracting bound [¹²⁵I]-E2 from added [¹²⁵I]-E2. Binding data was analysed by Hill-plots and by Scatchard plots (FIG. 11). The equilibrium binding results are shown in Table 1. The apparent K_d -values for [¹²⁵I]-E2 differed between the two ER:s with approximately a factor of four; $K_d(\text{hER}\alpha):K_d(\text{rER}\beta)=1:4$.

TABLE 1

Equilibrium dissociation constants for [¹²⁵ I]-E2 to the two subtypes.		
Receptor subtype	K_d (Hill-plot)	K_d (Scatchard-plot)
hER α	0.06 nM	0.09 nM
rER β	0.24 nM	0.42 nM

4. Competition experiments (IC_{50} determinations)

The experiments were performed as described above. IC_{50} values were obtained by applying a four parameter

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logistic analysis; $b=(b_{max}-b_{min})/(1+(I/IC_{50})^S)+b_{min}$, where I is the added concentration of binding inhibitor, IC_{50} is the concentration of inhibitor at half maximal binding and S is a slope factor. The free concentration of [¹²⁵I]-E2 was determined by sampling an aliquot from the wells at the end of the incubation and then subtract bound radioactivity from sampled total radioactivity.

Since the equilibrium binding experiments (above) showed that the K_d -values for [¹²⁵I]-E2 differed between the two ER:s, K_1 -values (from the Cheng-Prusoff equation: $K_1=IC_{50}/(1+L/K_d)$ where L is free ([¹²⁵I]-E2)) were calculated for the compounds investigated. Two approaches for calculating RBA (Relative Binding Affinity) were used. The RBA values were derived using either the IC_{50} values or the K_1 values. In both approaches, the value for the compound 16 α -bromo-estradiol was selected as the reference value (100%). Both approaches gave similar results. The results are summarized in FIG. 12. In these Figures “4-OH-Tam”=4-hydroxy-tamoxifen; “DES”=diethylstilbestrol; “Hexestr”=hexestrol; “ICI-164384”=ICI plc compound no. 164382; “17 β -E2”=17 β -estradiol; “16a-B-E2”=16 α -bromo-estradiol; “Ralox”=Raloxifen; and “17a-E2”=17 α diol.

The results show that ER α and ER β have significant different ligand binding affinities—the apparent K_d -values for [¹²⁵I]-E2 differed between the two ER’s by a factor of about 4 ($K_d(\text{hER}\alpha):K_d(\text{rER}\beta)\approx 1:4$). Some compounds investigated showed significant differences in the competition for binding of [¹²⁵I]-E2 to the ER’s. Certain compounds were found to be more potent inhibitors of [¹²⁵I]-E2 binding to hER α as compared to rER β whereas others were found to be more potent inhibitors of [¹²⁵I]-E2 binding to rER β than to hER α .

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 19

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2568 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Rattus rattus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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GGAATTCGG GGGAGCTGGC CCAGGGGAG CGGCTGGTGC TGCCACTGGC ATCCCTAGGC      60
ACCCAGGTCT GCAATAAAGT CTGGCAGCCA CTGCATGGCT GAGCGACAAC CAGTGGCTGG      120
GAGTCCGGCT CTGTGGCTGA GGAAAGCACC TGCTGCATT TAGAGAATGC AAAATAGAGA      180
ATGTTTACCT GCCAGTCATT ACATCTGAGT CCCATGAGTC TCTGAGAACA TAATGTCCAT      240
CTGTACCTCT TCTACAAGG AGTTTTCTCA GCTGCGACCC TCTGAAGACA TGGAGATCAA      300
AAACTCACCG TCGAGCCTTA GTTCCCTGCT TCCTATAACT GTAGCCAGTC CATCCTACCC      360
CTGGAGCACG GCCCATCTA CATCCCTTCC TCCTACGTAG ACAACCGCCA TGAGTATTCA      420
GCTATGACAT TCTACAGTCC TGCTGTGATG AACTACAGTG TTCCCGGCAG CACCAGTAAC      480
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-continued

CTGGACGGTG	GGCCTGTCCG	ACTGAGCACA	AGCCCAAATG	TGCTATGGCC	AACTTCTGGG	540
CACCTGTCTC	CTTTAGCGAC	CCATTGCCAA	TCATCGCTCC	TCTATGCAGA	ACCTCAAAAG	600
AGTCCTTGGT	GTGAAGCAAG	ATCACTAGAG	CACACCTTAC	CTGTAAACAG	AGAGACACTG	660
AAGAGGAAGC	TTAGTGGGAG	CAGTTGTGCC	AGCCCTGTTA	CTAGTCCAAA	CGCAAAGAGG	720
GATGCTCACT	TCTGCCCCGT	CTGCAGCGAT	TATGCATCTG	GGTATCATT	CGGCGTTTGG	780
TCATGTGAAG	GATGTAAGGC	CTTTTTTAAA	AGAAGCATTC	AAGGACATAA	TGATTATATC	840
TGTCCAGCCA	CGAATCAGTG	TACCATAGAC	AAGAACCGGC	GTAAAAGCTG	CCAGGCCTGC	900
CGACTTCGCA	AGTGTATGA	AGTAGGAATG	GTCAAGTGTG	GATCCAGGAG	AGAACGGTGT	960
GGGTACCGTA	TAGTGCGGAG	GCAGAGAAGT	TCTAGCGAGC	AGGTACACTG	CCTGAGCAAA	1020
GCCAAGAGAA	ACGGTGGGCA	TGCACCCCGG	GTGAAGGAGC	TACTGCTGAG	CACCTTGAGT	1080
CCAGAGCAAC	TGGTGCTCAC	CCTCCTGGAA	GCTGAACCAC	CCAATGTGCT	GGTGAGCCGT	1140
CCCAGCATGC	CCTTCACCGA	GGCCTCCATG	ATGATGTCCC	TACTAAGCT	GGCGGACAAG	1200
GAAGTGGTGC	ACATGATTGG	CTGGGCCAAG	AAAATCCCTG	GCTTTGTGGA	GCTCAGCCTG	1260
TTGGACCAAG	TCCGGCTCTT	AGAAAGCTGC	TGGATGGAGG	TGCTAATGGT	GGGACTGATG	1320
TGGCGCTCCA	TCGACCACC	CGGCAAGCTC	ATTTTCGCTC	CCGACCTCGT	TCTGGACAGG	1380
GATGAGGGGA	AGTGCGTAGA	AGGGATTCTG	GAAATCTTTG	ACATGCTCCT	GGCGACGACG	1440
TCAAGGTTCC	GTGAGTTAAA	ACTCCAGCAC	AAGGAGTATC	TCTGTGTGAA	GGCCATGATC	1500
CTCCTCAACT	CCAGTATGTA	CCCCTTGGCT	TCTGCAAACC	AGGAGGCAGA	AAGTAGCCGG	1560
AAGCTGACAC	ACCTACTGAA	CGCGGTGACA	GATGCCCTGG	TCTGGGTGAT	TGCGAAGAGT	1620
GGTATCTCCT	CCCAGCAGCA	GTCAGTCCGA	CTGGCCAACC	TCCTGATGCT	TCTTTCTCAC	1680
GTCAGGCACA	TCAGTAACAA	GGGCATGGAA	CATCTGCTCA	GCATGAAGTG	CAAAAATGTG	1740
GTCCCGGTGT	ATGACCTGCT	GCTGGAGATG	CTGAATGCTC	ACACGCTTCG	AGGGTACAAG	1800
TCCTCAATCT	CGGGGTCTGA	GTGCAGCTCA	ACAGAGGACA	GTAAGAACAA	AGAGAGCTCC	1860
CAGAACCTAC	AGTCTCAGTG	ATGGCCAGGC	CTGAGCGGGA	CAGACTACAG	AGATGGTCAA	1920
AAGTGAACA	TGTACCCTAG	CATCTGGGGG	TTCTCTTAG	GGCTGCCTTG	GTTACGCACC	1980
CCTTACCCAC	ACTGCACTTC	CCAGGAGTCA	GGGTGGTTGT	GTGGCGGTGT	TCCTCATAAC	2040
AGGATGTACC	ACCGAATGCC	AAGTTCTAAC	TTGTATAGCC	TTGAAGGCTC	TCGGTGTACT	2100
TACTTTCTGT	CTCCTTGCCC	ACTTGAAAAC	ATCTGAAAGG	TTCTGGAAC	AAAGGTCAAA	2160
GTCTGATTTG	GAAGGATTGT	CCTTAGTCAG	GAAAAGGAAT	ATGGCATGTG	ACACAGCTAT	2220
AAGAAATGGA	CTGTAGGACT	GTGTGGCCAT	AAAATCAACC	TTTGGATGGC	GTCTTCTAGA	2280
CCACTTGATT	GTAGGATTGA	AAACCACATT	GACAATCAGC	TCATTTGCA	TTCTGCCTC	2340
ACGGGTCTGT	GAGGACTCAT	TAATGTCATG	GGTTATTCTA	TCAAAGACCA	GAAAGATAGT	2400
GCAAGCTTAG	ATGTACCTTG	TTCTCCTCC	CAGACCCTTG	GGTTACATCC	TTAGAGCCTG	2460
CTTATTTGGT	CTGTCTGAAT	GTGGTCATTG	TCATGGGTTA	AGATTTAAAT	CTCTTTGTAA	2520
TATTGGCTTC	CTTGAAGCTA	TGTCATCTTT	CTCTCTCTCC	CGGAATTC		2568

(2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 485 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

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(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Rattus rattus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met	Thr	Phe	Tyr	Ser	Pro	Ala	Val	Met	Asn	Tyr	Ser	Val	Pro	Gly	Ser	1	5	10	15
Thr	Ser	Asn	Leu	Asp	Gly	Gly	Pro	Val	Arg	Leu	Ser	Thr	Ser	Pro	Asn	20	25	30	
Val	Leu	Trp	Pro	Thr	Ser	Gly	His	Leu	Ser	Pro	Leu	Ala	Thr	His	Cys	35	40	45	
Gln	Ser	Ser	Leu	Leu	Tyr	Ala	Glu	Pro	Gln	Lys	Ser	Pro	Trp	Cys	Glu	50	55	60	
Ala	Arg	Ser	Leu	Glu	His	Thr	Leu	Pro	Val	Asn	Arg	Glu	Thr	Leu	Lys	65	70	75	80
Arg	Lys	Leu	Ser	Gly	Ser	Ser	Cys	Ala	Ser	Pro	Val	Thr	Ser	Pro	Asn	85	90	95	
Ala	Lys	Arg	Asp	Ala	His	Phe	Cys	Pro	Val	Cys	Ser	Asp	Tyr	Ala	Ser	100	105	110	
Gly	Tyr	His	Tyr	Gly	Val	Trp	Ser	Cys	Glu	Gly	Cys	Lys	Ala	Phe	Phe	115	120	125	
Lys	Arg	Ser	Ile	Gln	Gly	His	Asn	Asp	Tyr	Ile	Cys	Pro	Ala	Thr	Asn	130	135	140	
Gln	Cys	Thr	Ile	Asp	Lys	Asn	Arg	Arg	Lys	Ser	Cys	Gln	Ala	Cys	Arg	145	150	155	160
Leu	Arg	Lys	Cys	Tyr	Glu	Val	Gly	Met	Val	Lys	Cys	Gly	Ser	Arg	Arg	165	170	175	
Glu	Arg	Cys	Gly	Tyr	Arg	Ile	Val	Arg	Arg	Gln	Arg	Ser	Ser	Ser	Glu	180	185	190	
Gln	Val	His	Cys	Leu	Ser	Lys	Ala	Lys	Arg	Asn	Gly	Gly	His	Ala	Pro	195	200	205	
Arg	Val	Lys	Glu	Leu	Leu	Leu	Ser	Thr	Leu	Ser	Pro	Glu	Gln	Leu	Val	210	215	220	
Leu	Thr	Leu	Leu	Glu	Ala	Glu	Pro	Pro	Asn	Val	Leu	Val	Ser	Arg	Pro	225	230	235	240
Ser	Met	Pro	Phe	Thr	Glu	Ala	Ser	Met	Met	Met	Ser	Leu	Thr	Lys	Leu	245	250	255	
Ala	Asp	Lys	Glu	Leu	Val	His	Met	Ile	Gly	Trp	Ala	Lys	Lys	Ile	Pro	260	265	270	
Gly	Phe	Val	Glu	Leu	Ser	Leu	Leu	Asp	Gln	Val	Arg	Leu	Leu	Glu	Ser	275	280	285	
Cys	Trp	Met	Glu	Val	Leu	Met	Val	Gly	Leu	Met	Trp	Arg	Ser	Ile	Asp	290	295	300	
His	Pro	Gly	Lys	Leu	Ile	Phe	Ala	Pro	Asp	Leu	Val	Leu	Asp	Arg	Asp	305	310	315	320
Glu	Gly	Lys	Cys	Val	Glu	Gly	Ile	Leu	Glu	Ile	Phe	Asp	Met	Leu	Leu	325	330	335	
Ala	Thr	Thr	Ser	Arg	Phe	Arg	Glu	Leu	Lys	Leu	Gln	His	Lys	Glu	Tyr	340	345	350	
Leu	Cys	Val	Lys	Ala	Met	Ile	Leu	Leu	Asn	Ser	Ser	Met	Tyr	Pro	Leu	355	360	365	
Ala	Ser	Ala	Asn	Gln	Glu	Ala	Glu	Ser	Ser	Arg	Lys	Leu	Thr	His	Leu	370	375	380	
Leu	Asn	Ala	Val	Thr	Asp	Ala	Leu	Val	Trp	Val	Ile	Ala	Lys	Ser	Gly	385	390	395	400

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Ile Ser Ser Gln Gln Gln Ser Val Arg Leu Ala Asn Leu Leu Met Leu
 405 410 415

Leu Ser His Val Arg His Ile Ser Asn Lys Gly Met Glu His Leu Leu
 420 425 430

Ser Met Lys Cys Lys Asn Val Val Pro Val Tyr Asp Leu Leu Leu Glu
 435 440 445

Met Leu Asn Ala His Thr Leu Arg Gly Tyr Lys Ser Ser Ile Ser Gly
 450 455 460

Ser Glu Cys Ser Ser Thr Glu Asp Ser Lys Asn Lys Glu Ser Ser Gln
 465 470 475 480

Asn Leu Gln Ser Gln
 485

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 485 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Thr Phe Tyr Ser Pro Ala Val Met Asn Tyr Ser Ile Pro Ser Asn
 1 5 10 15

Val Thr Asn Leu Glu Gly Gly Pro Gly Arg Gln Thr Thr Ser Pro Asn
 20 25 30

Val Leu Trp Pro Thr Pro Gly His Leu Ser Pro Leu Val Val His Arg
 35 40 45

Gln Leu Ser His Leu Tyr Ala Glu Pro Gln Lys Ser Pro Trp Cys Glu
 50 55 60

Ala Arg Ser Leu Glu His Thr Leu Pro Val Asn Arg Glu Thr Leu Lys
 65 70 75 80

Arg Lys Val Ser Gly Asn Arg Cys Ala Ser Pro Val Thr Gly Pro Gly
 85 90 95

Ser Lys Arg Asp Ala His Phe Cys Ala Val Cys Ser Asp Tyr Ala Ser
 100 105 110

Gly Tyr His Tyr Gly Val Trp Ser Cys Glu Gly Cys Lys Ala Phe Phe
 115 120 125

Lys Arg Ser Ile Gln Gly His Asn Asp Tyr Ile Cys Pro Ala Thr Asn
 130 135 140

Gln Cys Thr Ile Asp Lys Asn Arg Arg Lys Ser Cys Gln Ala Cys Arg
 145 150 155 160

Leu Arg Lys Cys Tyr Glu Val Gly Met Val Lys Cys Gly Ser Arg Arg
 165 170 175

Glu Arg Cys Gly Tyr Arg Leu Val Arg Arg Gln Arg Ser Ala Asp Glu
 180 185 190

Gln Leu His Cys Ala Gly Lys Ala Lys Arg Ser Gly Gly His Ala Pro
 195 200 205

Arg Val Arg Glu Leu Leu Leu Asp Ala Leu Ser Pro Glu Gln Leu Val
 210 215 220

Leu Thr Leu Leu Glu Ala Glu Pro Pro His Val Leu Ile Ser Arg Pro
 225 230 235 240

Ser Ala Pro Phe Thr Glu Ala Ser Met Met Met Ser Leu Thr Lys Leu
 245 250 255

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Ala Asp Lys Glu Leu Val His Met Ile Ser Trp Ala Lys Lys Ile Pro
 260 265 270

Gly Phe Val Glu Leu Ser Leu Phe Asp Gln Val Arg Leu Leu Glu Ser
 275 280 285

Cys Trp Met Glu Val Leu Met Met Gly Leu Met Trp Arg Ser Ile Asp
 290 295 300

His Pro Gly Lys Leu Ile Phe Ala Pro Asp Leu Val Leu Asp Arg Asp
 305 310 315 320

Glu Gly Lys Cys Val Glu Gly Ile Leu Glu Ile Phe Asp Met Leu Leu
 325 330 335

Ala Thr Thr Ser Arg Phe Arg Glu Leu Lys Leu Gln His Lys Glu Tyr
 340 345 350

Leu Cys Val Lys Ala Met Ile Leu Leu Asn Ser Ser Met Tyr Pro Leu
 355 360 365

Val Thr Ala Thr Gln Asp Ala Asp Ser Ser Arg Lys Leu Ala His Leu
 370 375 380

Leu Asn Ala Val Thr Asp Ala Leu Val Trp Val Ile Ala Lys Ser Gly
 385 390 395 400

Ile Ser Ser Gln Gln Gln Ser Met Arg Leu Ala Asn Leu Leu Met Leu
 405 410 415

Leu Ser His Val Arg His Ala Ser Asn Lys Gly Met Glu His Leu Leu
 420 425 430

Asn Met Lys Cys Lys Asn Val Val Pro Val Tyr Asp Leu Leu Leu Glu
 435 440 445

Met Leu Asn Ala His Val Leu Arg Gly Cys Lys Ser Ser Ile Thr Gly
 450 455 460

Ser Glu Cys Ser Pro Ala Glu Asp Ser Lys Ser Lys Glu Gly Ser Gln
 465 470 475 480

Asn Leu Gln Ser Gln
 485

(2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1460 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CTATGACATT CTACAGTCCT GCTGTGATGA ATTACAGCAT TCCCAGCAAT GTCACAACT 60

TGGAAGGTGG GCCTGGTCGG CAGACCACAA GCCCAAATGT GTTGTGGCCA ACACCTGGGC 120

ACCTTTCTCC TTTAGTGGTC CATCGCCAGT TATCACATCT GTATGCGGAA CCTCAAAGA 180

GTCCTGGTG TGAAGCAAGA TCGCTAGAAC ACACCTTACC TGTAACAGA GAGACTGA 240

AAAGGAAGGT TAGTGGGAAC CGTTGCGCCA GCCCTGTTAC TGGTCCAGGT TCAAAGAGGG 300

ATGCTCACTT CTGCGCTGTC TGCAGCGATT ACGCATCGGG ATATCACTAT GGAGTCTGGT 360

CGTGTGAAGG ATGTAAGGCC TTTTTTAAAA GAAGCATTCA AGGACATAAT GATTATATTT 420

GTCCAGCTAC AAATCAGTGT ACAATCGATA AAAACCGGCG CAAGAGCTGC CAGGCCTGCC 480

GACTTCGGAA GTGTTACGAA GTGGGAATGG TGAAGTGTGG CTCCCGGAGA GAGAGATGTG 540

GGTACCGCCT TGTGCGGAGA CAGAGAAGTG CCGACGAGCA GCTGCACTGT GCCGGCAAGG 600

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CCAAGAGAAG TGGCGGCCAC GCGCCCCGAG TGCGGGAGCT GCTGCTGGAC GCCCTGAGCC 660
 CCGAGCAGCT AGTGCTCACC CTCCTGGAGG CTGAGCCGCC CCATGTGCTG ATCAGCCGCC 720
 CCAGTGCGCC CTTCAACGAG GCCTCCATGA TGATGTCCCT GACCAAGTTG GCCGACAAGG 780
 AGTTGGTACA CATGATCAGC TGGGCCAAGA AGATTCCCGG CTTTGTGGAG CTCAGCCTGT 840
 TCGACCAAGT GCGGCTCTTG GAGAGCTGTT GGATGGAGGT GTTAATGATG GGGCTGATGT 900
 GCGCTCAAT TGACCACCCC GGCAAGCTCA TCTTTGCTCC AGATCTTGTT CTGGACAGGG 960
 ATGAGGGGAA ATGCGTAGAA GGAATTCTGG AAATCTTTGA CATGCTCCTG GCAACTACTT 1020
 CAAGGTTTCG AGAGTTAAAA CTCCAACACA AAGAATATCT CTGTGTCAAG GCCATGATCC 1080
 TGCTCAATTC CAGTATGTAC CCTCTGGTCA CAGCGACCCA GGATGCTGAC AGCAGCCGGA 1140
 AGCTGGCTCA CTTGCTGAAC GCCGTGACCG ATGCTTTGGT TTGGGTGATT GCCAAGAGCG 1200
 GCATCTCCTC CCAGCAGCAA TCCATGCGCC TGGCTAACCT CCTGATGCTC CTGTCCCACG 1260
 TCAGGCATGC GAGTAACAAG GGCATGGAAC ATCTGCTCAA CATGAAGTGC AAAAATGTGG 1320
 TCCCAGTGTA TGACCTGCTG CTGGAGATGC TGAATGCCCA CGTGCTTCGC GGGTGCAAGT 1380
 CCTCCATCAC GGGGTCCGAG TGCAGCCCGG CAGAGGACAG TAAAAGCAAA GAGGGCTCCC 1440
 AGAACCTACA GTCTCAGTGA 1460

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 485 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Ala Phe Tyr Ser Pro Ala Val Met Asn Tyr Ser Val Pro Ser Ser
 1 5 10 15
 Thr Gly Asn Leu Glu Gly Gly Pro Val Arg Gln Thr Ala Ser Pro Asn
 20 25 30
 Val Leu Trp Pro Thr Ser Gly His Leu Ser Pro Leu Ala Thr His Cys
 35 40 45
 Gln Ser Ser Leu Leu Tyr Ala Glu Pro Gln Lys Ser Pro Trp Cys Glu
 50 55 60
 Ala Arg Ser Leu Glu His Thr Leu Pro Val Asn Arg Glu Thr Leu Lys
 65 70 75 80
 Arg Lys Leu Gly Gly Ser Gly Cys Ala Ser Pro Val Thr Ser Pro Ser
 85 90 95
 Thr Lys Arg Asp Ala His Phe Cys Ala Val Cys Ser Asp Tyr Ala Ser
 100 105 110
 Gly Tyr His Tyr Gly Val Trp Ser Cys Glu Gly Cys Lys Ala Phe Phe
 115 120 125
 Lys Arg Ser Ile Gln Gly His Asn Asp Tyr Ile Cys Pro Ala Thr Asn
 130 135 140
 Gln Cys Thr Ile Asp Lys Asn Arg Arg Lys Asn Cys Gln Ala Cys Arg
 145 150 155 160
 Leu Arg Lys Cys Tyr Glu Val Gly Met Val Lys Cys Gly Ser Arg Arg
 165 170 175
 Glu Arg Cys Gly Tyr Arg Ile Val Arg Arg Gln Arg Ser Ala Ser Glu
 180 185 190

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Gln Val His Cys Leu Asn Lys Ala Lys Arg Thr Ser Gly His Thr Pro
 195 200 205
 Arg Val Lys Glu Leu Leu Leu Asn Ser Leu Ser Pro Glu Gln Leu Val
 210 215 220
 Leu Thr Leu Leu Glu Ala Glu Pro Pro Asn Val Leu Val Ser Arg Pro
 225 230 235 240
 Ser Met Pro Phe Thr Glu Ala Ser Met Met Met Ser Leu Thr Lys Leu
 245 250 255
 Ala Asp Lys Glu Leu Val His Met Ile Gly Trp Ala Lys Lys Ile Pro
 260 265 270
 Gly Phe Val Glu Leu Ser Leu Leu Asp Gln Val Arg Leu Leu Glu Ser
 275 280 285
 Cys Trp Met Glu Val Leu Met Val Gly Leu Met Trp Arg Ser Ile Asp
 290 295 300
 His Pro Gly Lys Leu Ile Phe Ala Pro Asp Leu Val Leu Asp Arg Asp
 305 310 315 320
 Glu Gly Lys Cys Val Glu Gly Ile Leu Glu Ile Phe Asp Met Leu Leu
 325 330 335
 Ala Thr Thr Ala Arg Phe Arg Glu Leu Lys Leu Gln His Lys Glu Tyr
 340 345 350
 Leu Cys Val Lys Ala Met Ile Leu Leu Asn Ser Ser Met Tyr His Leu
 355 360 365
 Ala Thr Ala Ser Gln Glu Ala Glu Ser Ser Arg Lys Leu Thr His Leu
 370 375 380
 Leu Asn Ala Val Thr Asp Ala Leu Val Trp Val Ile Ser Lys Ser Arg
 385 390 395 400
 Ile Ser Ser Gln Gln Gln Ser Val Arg Leu Ala Asn Leu Leu Met Leu
 405 410 415
 Leu Ser His Val Arg His Ile Ser Asn Lys Gly Met Glu His Leu Leu
 420 425 430
 Ser Met Lys Cys Lys Asn Val Val Pro Val Tyr Asp Leu Leu Leu Glu
 435 440 445
 Met Leu Asn Ala His Thr Leu Arg Gly Tyr Lys Ser Ser Ile Ser Gly
 450 455 460
 Ser Gly Cys Cys Ser Thr Glu Asp Ser Lys Ser Lys Glu Gly Ser Gln
 465 470 475 480
 Asn Leu Gln Ser Gln
 485

(2) INFORMATION FOR SEQ ID NO: 6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1458 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Mus musculus

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ATGGCATTCT ACAGTCCTGC TGTGATGAAC TACAGTGTTC CCAGCAGCAC CGGTAACCTG 60
 GAAGGTGGGC CTGTTGCGCA GACTGCAAGC CCAAATGTGC TATGGCCAAC TTCTGGACAC 120
 CTCTCTCCTT TAGCCACCCA CTGCCAATCA TCGCTTCTCT ATGCAGAACC TCAAAAGAGT 180
 CCTTGGTGTG AAGCAAGATC ACTAGAACAC ACCTTGCCCTG TAAACAGAGA GACCCTGAAG 240

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AGGAAGCTTG GCGGGAGCGG TTGTGCCAGC CCTGTTACTA GTCCAAGCAC CAAGAGGGAT 300
 GCTCACTTCT GTGCCGTCG CAGTGATTAT GCATCTGGGT ATCATTACGG TGTCTGGTCC 360
 TGTGAAGGAT GTAAGGCCCT TTTTAAAAGA AGCATTCAAG GACATAATGA CTATATCTGT 420
 CCAGCCACGA ATCAGTGTAC GATAGACAAG AACCGGCGTA AAAACTGCCA GGCCTGCCGA 480
 CTTCGCAAGT GTTACGAAGT AGGAATGGTC AAGTGTGGAT CCAGGAGAGA AAGGTGTGGG 540
 TACCGAATAG TACGAAGACA GAGAAGTGCC AGCGAGCAGG TGCATTGCCT GAACAAAGCC 600
 AAGAGAACCA GTGGGCACAC ACCCCGGGTG AAGGAGCTAC TGCTGAACTC TCTGAGTCCC 660
 GAGCAGCTGG TGCTCACCTT GCTGGAAGCT GAGCCACCCA ATGTGCTAGT GAGTCGTCCC 720
 AGCATGCCCT TCACCGAGGC CTCCATGATG ATGTCCCTTA CGAAGCTGGC TGACAAGGAA 780
 CTGGTGCACA TGATTGGCTG GGCCAAGAAA ATCCCTGGCT TTGTGGAGCT CAGCCTGTTG 840
 GACCAAGTCC GCCTCTTGA AAGCTGCTGG ATGGAGGTGC TGATGGTGGG GCTGATGTGG 900
 CGCTCCATCG ACCACCCCGG CAAGCTCATC TTTGCTCCAG ACCTCGTTCT GGACAGGGAT 960
 GAGGGGAAGT GCGTGAAGG GATTCTGGAA ATCTTTGACA TGCTCCTGGC GACGACGGCA 1020
 CGGTTCCGTG AGTTAAAAC GCAGCACAAA GAATATCTGT GTGTGAAGGC CATGATTCTC 1080
 CTCAACTCCA GTATGTACCA CTTGGCTACC GCAAGCCAGG AAGCAGAGAG TAGCCGGAAG 1140
 CTGACACACC TATTGAACGC AGTGACAGAT GCCCTGGTCT GGGTGATTTC GAAGAGTAGA 1200
 ATCTCTTCCC AGCAGCAGTC AGTCCGTCTG GCCAACCTCC TGATGCTTCT TTCTCATGTC 1260
 AGGCACATCA GTAACAAGG CATGGAACAT CTGCTCAGCA TGAAGTGCAA AAATGTGGTC 1320
 CCGGTGTACG ACCTGCTGCT GGAGATGCTG AATGCTCACA CGCTTCGAGG GTACAAGTCC 1380
 TCAATCTCGG GGTCTGGGTG CTGCTCGACA GAGGACAGTA AGAGCAAAGA GGGCTCCCAG 1440
 AACCTCCAGT CTCAGTGA 1458

(2) INFORMATION FOR SEQ ID NO: 7:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 226 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Rattus rattus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Glu Leu Val His Met Ile Gly Trp Ala Lys Lys Ile Pro Gly Phe Val
 1 5 10 15
 Glu Leu Ser Leu Leu Asp Gln Val Arg Leu Leu Glu Ser Cys Trp Met
 20 25 30
 Glu Val Leu Met Val Gly Leu Met Trp Arg Ser Ile Asp His Pro Gly
 35 40 45
 Lys Leu Ile Phe Ala Pro Asp Leu Val Leu Asp Arg Asp Glu Gly Lys
 50 55 60
 Cys Val Glu Gly Ile Leu Glu Ile Phe Asp Met Leu Leu Ala Thr Thr
 65 70 75 80
 Ser Arg Phe Arg Glu Leu Lys Leu Gln His Lys Glu Tyr Leu Cys Val
 85 90 95
 Lys Ala Met Ile Leu Leu Asn Ser Ser Met Tyr Pro Leu Ala Ser Ala
 100 105 110
 Asn Gln Glu Ala Glu Ser Ser Arg Lys Leu Thr His Leu Leu Asn Ala
 115 120 125

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Val Thr Asp Ala Leu Val Trp Val Ile Ala Lys Ser Gly Ile Ser Ser
 130 135 140

Gln Gln Gln Ser Val Arg Leu Ala Asn Leu Leu Met Leu Leu Ser His
 145 150 155 160

Val Arg His Ile Ser Asn Lys Gly Met Glu His Leu Leu Ser Met Lys
 165 170 175

Cys Lys Asn Val Val Pro Val Tyr Asp Leu Leu Leu Glu Met Leu Asn
 180 185 190

Ala His Thr Leu Arg Gly Tyr Lys Ser Ser Ile Ser Gly Ser Glu Cys
 195 200 205

Ser Ser Thr Glu Asp Ser Lys Asn Lys Glu Ser Ser Gln Asn Leu Gln
 210 215 220

Ser Gln
 225

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 243 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Rattus rattus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Glu Leu Val His Met Ile Asn Trp Ala Lys Arg Val Pro Gly Phe Gly
 1 5 10 15

Asp Leu Asn Leu His Asp Gln Val His Leu Leu Glu Cys Ala Trp Leu
 20 25 30

Glu Ile Leu Met Ile Gly Leu Val Trp Arg Ser Met Glu His Pro Gly
 35 40 45

Lys Leu Leu Phe Ala Pro Asn Leu Leu Leu Asp Arg Asn Gln Gly Lys
 50 55 60

Cys Val Glu Gly Met Val Glu Ile Phe Asp Met Leu Leu Ala Thr Ser
 65 70 75 80

Ser Arg Phe Arg Met Met Asn Leu Gln Gly Glu Glu Phe Val Cys Leu
 85 90 95

Lys Ser Ile Ile Leu Leu Asn Ser Gly Val Tyr Thr Phe Leu Ser Ser
 100 105 110

Thr Leu Lys Ser Leu Glu Glu Lys Asp His Ile His Arg Val Leu Asp
 115 120 125

Lys Ile Asn Asp Thr Leu Ile His Leu Met Ala Lys Ala Gly Leu Thr
 130 135 140

Leu Gln Gln Gln His Arg Arg Leu Ala Gln Leu Leu Leu Ile Leu Ser
 145 150 155 160

His Ile Arg His Met Ser Asn Lys Gly Met Glu His Leu Tyr Asn Met
 165 170 175

Lys Cys Lys Asn Val Val Pro Leu Tyr Asp Leu Leu Leu Glu Met Leu
 180 185 190

Asp Ala His Arg Leu His Ala Pro Ala Ser Arg Met Gly Val Pro Pro
 195 200 205

Glu Glu Pro Ser Gln Ser Gln Leu Thr Thr Thr Ser Ser Thr Ser Ala
 210 215 220

His Ser Leu Gln Thr Tyr Tyr Ile Pro Pro Glu Ala Glu Gly Phe Pro
 225 230 235 240

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Asn Thr Ile

(2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 243 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Glu Leu Val His Met Ile Asn Trp Ala Lys Arg Val Pro Gly Phe Gly
 1 5 10 15
 Asp Leu Asn Leu His Asp Gln Val His Leu Leu Glu Cys Ala Trp Leu
 20 25 30
 Glu Ile Leu Met Ile Gly Leu Val Trp Arg Ser Met Glu His Pro Gly
 35 40 45
 Lys Leu Leu Phe Ala Pro Asn Leu Leu Leu Asp Arg Asn Gln Gly Lys
 50 55 60
 Cys Val Glu Gly Met Val Glu Ile Phe Asp Met Leu Leu Ala Thr Ser
 65 70 75 80
 Ser Arg Phe Arg Met Met Asn Leu Gln Gly Glu Glu Phe Val Cys Leu
 85 90 95
 Lys Ser Ile Ile Leu Leu Asn Ser Gly Val Tyr Thr Phe Leu Ser Ser
 100 105 110
 Thr Leu Lys Ser Leu Glu Glu Lys Asp His Ile His Arg Val Leu Asp
 115 120 125
 Lys Ile Thr Asp Thr Leu Ile His Leu Met Ala Lys Ala Gly Leu Thr
 130 135 140
 Leu Gln Gln Gln His Arg Arg Leu Ala Gln Leu Leu Leu Ile Leu Ser
 145 150 155 160
 His Ile Arg His Met Ser Asn Lys Gly Met Glu His Leu Tyr Asn Met
 165 170 175
 Lys Cys Lys Asn Val Val Pro Leu Tyr Asp Leu Leu Leu Glu Met Leu
 180 185 190
 Asp Ala His Arg Leu His Ala Pro Ala Ser Arg Met Gly Val Pro Pro
 195 200 205
 Glu Glu Pro Ser Gln Thr Gln Leu Ala Thr Thr Ser Ser Thr Ser Ala
 210 215 220
 His Ser Leu Gln Thr Tyr Tyr Ile Pro Pro Glu Ala Glu Gly Phe Pro
 225 230 235 240

Asn Thr Ile

(2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 243 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Glu Leu Val His Met Ile Asn Trp Ala Lys Arg Val Pro Gly Phe Val
 1 5 10 15

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Asp Leu Thr Leu His Asp Gln Val His Leu Leu Glu Cys Ala Trp Leu
 20 25 30
 Glu Ile Leu Met Ile Gly Leu Val Trp Arg Ser Met Glu His Pro Val
 35 40 45
 Lys Leu Leu Phe Ala Pro Asn Leu Leu Leu Asp Arg Asn Gln Gly Lys
 50 55 60
 Cys Val Glu Gly Met Val Glu Ile Phe Asp Met Leu Leu Ala Thr Ser
 65 70 75 80
 Ser Arg Phe Arg Met Met Asn Leu Gln Gly Glu Glu Phe Val Cys Leu
 85 90 95
 Lys Ser Ile Ile Leu Leu Asn Ser Gly Val Tyr Thr Phe Leu Ser Ser
 100 105 110
 Thr Leu Lys Ser Leu Glu Glu Lys Asp His Ile His Arg Val Leu Asp
 115 120 125
 Lys Ile Thr Asp Thr Leu Ile His Leu Met Ala Lys Ala Gly Leu Thr
 130 135 140
 Leu Gln Gln Gln His Gln Arg Leu Ala Gln Leu Leu Leu Ile Leu Ser
 145 150 155 160
 His Ile Arg His Met Ser Asn Lys Gly Met Glu His Leu Tyr Ser Met
 165 170 175
 Lys Cys Lys Asn Val Val Pro Leu Tyr Asp Leu Leu Leu Glu Met Leu
 180 185 190
 Asp Ala His Arg Leu His Ala Pro Thr Ser Arg Gly Gly Ala Ser Val
 195 200 205
 Glu Glu Thr Asp Gln Ser His Leu Ala Thr Ala Gly Ser Thr Ser Ser
 210 215 220
 His Ser Leu Gln Lys Tyr Tyr Ile Thr Gly Glu Ala Glu Gly Phe Pro
 225 230 235 240
 Ala Thr Val

(2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 66 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Rattus rattus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Cys Pro Val Cys Ser Asp Tyr Ala Ser Gly Tyr His Tyr Gly Val Trp
 1 5 10 15
 Ser Cys Glu Gly Cys Lys Ala Phe Phe Lys Arg Ser Ile Gln Gly His
 20 25 30
 Asn Asp Tyr Ile Cys Pro Ala Thr Asn Gln Cys Thr Ile Asp Lys Asn
 35 40 45
 Arg Arg Lys Ser Cys Gln Ala Cys Arg Leu Arg Lys Cys Tyr Glu Val
 50 55 60
 Gly Met
 65

(2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 66 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Cys Ala Val Cys Asn Asp Tyr Ala Ser Gly Tyr His Tyr Gly Val Trp
 1 5 10 15
 Ser Cys Glu Gly Cys Lys Ala Phe Phe Lys Arg Ser Ile Gln Gly His
 20 25 30
 Asn Asp Tyr Met Cys Pro Ala Thr Asn Gln Cys Thr Ile Asp Lys Asn
 35 40 45
 Arg Arg Lys Ser Cys Gln Ala Cys Arg Leu Arg Lys Cys Tyr Glu Val
 50 55 60
 Gly Met
 65

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 484 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Rattus rattus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Met Thr Phe Tyr Ser Pro Ala Val Met Asn Tyr Ser Val Pro Gly Ser
 1 5 10 15
 Thr Ser Asn Leu Asp Gly Gly Pro Val Arg Leu Ser Thr Ser Pro Asn
 20 25 30
 Val Leu Trp Pro Thr Ser Gly His Leu Ser Pro Leu Ala Thr His Cys
 35 40 45
 Gln Ser Ser Leu Leu Tyr Ala Glu Pro Gln Lys Ser Pro Trp Cys Glu
 50 55 60
 Ala Arg Ser Leu Glu His Thr Leu Pro Val Asn Arg Glu Thr Leu Lys
 65 70 75 80
 Arg Lys Leu Ser Gly Ser Ser Cys Ala Ser Pro Val Thr Ser Pro Asn
 85 90 95
 Ala Lys Arg Asp Ala His Phe Cys Pro Val Cys Ser Asp Tyr Ala Ser
 100 105 110
 Gly Tyr His Tyr Gly Val Trp Ser Cys Glu Gly Cys Lys Ala Phe Phe
 115 120 125
 Lys Arg Ser Ile Gln Gly His Asn Asp Tyr Ile Cys Pro Ala Thr Asn
 130 135 140
 Gln Cys Thr Ile Asp Lys Asn Arg Arg Lys Ser Cys Gln Ala Cys Arg
 145 150 155 160
 Leu Arg Lys Cys Tyr Glu Val Gly Met Val Lys Cys Gly Ser Arg Arg
 165 170 175
 Glu Arg Cys Gly Tyr Arg Ile Val Arg Arg Gln Arg Ser Ser Ser Glu
 180 185 190
 Gln Val His Cys Leu Ser Lys Ala Lys Arg Asn Gly Gly His Ala Pro
 195 200 205
 Arg Val Lys Glu Leu Leu Leu Ser Thr Leu Ser Pro Glu Gln Leu Val
 210 215 220
 Leu Thr Leu Leu Glu Ala Glu Pro Pro Asn Val Leu Val Ser Arg Pro
 225 230 235 240
 Ser Met Pro Phe Thr Glu Ala Ser Met Met Met Ser Leu Thr Lys Leu
 245 250 255

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Ala Asp Lys Glu Leu Val His Met Ile Gly Trp Ala Lys Lys Ile Pro
 260 265 270

Gly Phe Val Glu Leu Ser Leu Leu Asp Gln Val Arg Leu Leu Glu Ser
 275 280 285

Cys Trp Met Glu Val Leu Met Val Gly Leu Met Trp Arg Ser Ile Asp
 290 295 300

His Pro Gly Lys Leu Ile Phe Ala Pro Asp Leu Val Leu Asp Arg Asp
 305 310 315 320

Glu Gly Lys Cys Val Glu Gly Ile Leu Glu Ile Phe Asp Met Leu Leu
 325 330 335

Ala Thr Thr Ser Arg Phe Arg Glu Leu Lys Leu Gln His Lys Glu Tyr
 340 345 350

Leu Cys Val Lys Ala Met Ile Leu Leu Asn Ser Ser Met Tyr Pro Leu
 355 360 365

Ala Ser Ala Asn Gln Glu Ala Glu Ser Ser Arg Lys Leu Thr His Leu
 370 375 380

Leu Asn Ala Val Thr Asp Ala Leu Val Trp Val Ile Ala Lys Ser Gly
 385 390 395 400

Ile Ser Ser Gln Gln Gln Ser Val Arg Leu Ala Asn Leu Leu Met Leu
 405 410 415

Leu Ser His Val Arg His Ile Ser Asn Lys Gly Met Glu His Leu Leu
 420 425 430

Ser Met Lys Cys Lys Asn Val Val Pro Val Tyr Asp Leu Leu Leu Glu
 435 440 445

Met Leu Asn Ala His Thr Leu Arg Gly Tyr Lys Ser Ser Ile Ser Gly
 450 455 460

Ser Glu Cys Ser Ser Thr Glu Asp Ser Lys Asn Lys Glu Ser Ser Gln
 465 470 475 480

Asn Leu Gln Ser

(2) INFORMATION FOR SEQ ID NO: 14:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 484 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Met Ala Phe Tyr Ser Pro Ala Val Met Asn Tyr Ser Val Pro Ser Ser
 1 5 10 15

Thr Gly Asn Leu Glu Gly Gly Pro Val Arg Gln Thr Ala Ser Pro Asn
 20 25 30

Val Leu Trp Pro Thr Ser Gly His Leu Ser Pro Leu Ala Thr His Cys
 35 40 45

Gln Ser Ser Leu Leu Tyr Ala Glu Pro Gln Lys Ser Pro Trp Cys Glu
 50 55 60

Ala Arg Ser Leu Glu His Thr Leu Pro Val Asn Arg Glu Thr Leu Lys
 65 70 75 80

Arg Lys Leu Gly Gly Ser Gly Cys Ala Ser Pro Val Thr Ser Pro Ser
 85 90 95

Thr Lys Arg Asp Ala His Phe Cys Ala Val Cys Ser Asp Tyr Ala Ser
 100 105 110

Gly Tyr His Tyr Gly Val Trp Ser Cys Glu Gly Cys Lys Ala Phe Phe

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115				120				125							
Lys	Arg	Ser	Ile	Gln	Gly	His	Asn	Asp	Tyr	Ile	Cys	Pro	Ala	Thr	Asn
	130					135					140				
Gln	Cys	Thr	Ile	Asp	Lys	Asn	Arg	Arg	Lys	Asn	Cys	Gln	Ala	Cys	Arg
145					150				155						160
Leu	Arg	Lys	Cys	Tyr	Glu	Val	Gly	Met	Val	Lys	Cys	Gly	Ser	Arg	Arg
				165					170					175	
Glu	Arg	Cys	Gly	Tyr	Arg	Ile	Val	Arg	Arg	Gln	Arg	Ser	Ala	Ser	Glu
			180					185					190		
Gln	Val	His	Cys	Leu	Asn	Lys	Ala	Lys	Arg	Thr	Ser	Gly	His	Thr	Pro
		195					200					205			
Arg	Val	Lys	Glu	Leu	Leu	Leu	Asn	Ser	Leu	Ser	Pro	Glu	Gln	Leu	Val
	210						215				220				
Leu	Thr	Leu	Leu	Glu	Ala	Glu	Pro	Pro	Asn	Val	Leu	Val	Ser	Arg	Pro
225					230					235					240
Ser	Met	Pro	Phe	Thr	Glu	Ala	Ser	Met	Met	Met	Ser	Leu	Thr	Lys	Leu
				245					250					255	
Ala	Asp	Lys	Glu	Leu	Val	His	Met	Ile	Gly	Trp	Ala	Lys	Lys	Ile	Pro
			260					265						270	
Gly	Phe	Val	Glu	Leu	Ser	Leu	Leu	Asp	Gln	Val	Arg	Leu	Leu	Glu	Ser
		275					280					285			
Cys	Trp	Met	Glu	Val	Leu	Met	Val	Gly	Leu	Met	Trp	Arg	Ser	Ile	Asp
	290					295					300				
His	Pro	Gly	Lys	Leu	Ile	Phe	Ala	Pro	Asp	Leu	Val	Leu	Asp	Arg	Asp
305					310					315					320
Glu	Gly	Lys	Cys	Val	Glu	Gly	Ile	Leu	Glu	Ile	Phe	Asp	Met	Leu	Leu
				325					330					335	
Ala	Thr	Thr	Ala	Arg	Phe	Arg	Glu	Leu	Lys	Leu	Gln	His	Lys	Glu	Tyr
			340					345					350		
Leu	Cys	Val	Lys	Ala	Met	Ile	Leu	Leu	Asn	Ser	Ser	Met	Tyr	His	Leu
		355					360					365			
Ala	Thr	Ala	Ser	Gln	Glu	Ala	Glu	Ser	Ser	Arg	Lys	Leu	Thr	His	Leu
		370				375					380				
Leu	Asn	Ala	Val	Thr	Asp	Ala	Leu	Val	Trp	Val	Ile	Ser	Lys	Ser	Arg
385					390					395					400
Ile	Ser	Ser	Gln	Gln	Gln	Ser	Val	Arg	Leu	Ala	Asn	Leu	Leu	Met	Leu
			405						410					415	
Leu	Ser	His	Val	Arg	His	Ile	Ser	Asn	Lys	Gly	Met	Glu	His	Leu	Leu
			420					425					430		
Ser	Met	Lys	Cys	Lys	Asn	Val	Val	Pro	Val	Tyr	Asp	Leu	Leu	Leu	Glu
		435					440					445			
Met	Leu	Asn	Ala	His	Thr	Leu	Arg	Gly	Tyr	Lys	Ser	Ser	Ile	Ser	Gly
	450					455					460				
Ser	Gly	Cys	Cys	Ser	Thr	Glu	Asp	Ser	Lys	Ser	Lys	Glu	Gly	Ser	Gln
465					470					475					480
Asn	Leu	Gln	Ser												

(2) INFORMATION FOR SEQ ID NO: 15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 384 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:

-continued

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Ala Leu Ser Pro Leu Val Val His Arg Gln Leu Ser His Leu Tyr Ala
 1 5 10 15
 Glu Pro Gln Lys Ser Pro Trp Cys Glu Ala Arg Ser Leu Glu His Thr
 20 25 30
 Leu Pro Val Asn Arg Glu Thr Leu Lys Arg Lys Val Ser Gly Asn Arg
 35 40 45
 Cys Ala Ser Pro Val Thr Gly Pro Gly Ser Lys Arg Asp Ala His Phe
 50 55 60
 Cys Ala Val Cys Ser Asp Tyr Ala Ser Gly Tyr His Tyr Gly Val Trp
 65 70 75 80
 Ser Cys Glu Gly Cys Lys Ala Phe Phe Lys Arg Ser Ile Gln Gly His
 85 90 95
 Asn Asp Tyr Ile Cys Pro Ala Thr Asn Gln Cys Thr Ile Asp Lys Asn
 100 105 110
 Arg Arg Lys Ser Cys Gln Ala Cys Arg Leu Arg Lys Cys Tyr Glu Val
 115 120 125
 Gly Met Val Lys Cys Gly Ser Arg Arg Glu Arg Cys Gly Tyr Arg Leu
 130 135 140
 Val Arg Arg Gln Arg Ser Ala Asp Glu Gln Leu His Cys Ala Gly Lys
 145 150 155 160
 Ala Lys Arg Ser Gly Gly His Ala Pro Arg Val Arg Glu Leu Leu Leu
 165 170 175
 Asp Ala Leu Ser Pro Glu Gln Leu Val Leu Thr Leu Leu Glu Ala Glu
 180 185 190
 Pro Pro His Val Leu Ile Ser Arg Pro Ser Ala Pro Phe Thr Glu Ala
 195 200 205
 Ser Met Met Met Ser Leu Thr Lys Leu Ala Asp Lys Glu Leu Val His
 210 215 220
 Met Ile Ser Trp Ala Lys Lys Ile Pro Gly Phe Val Glu Leu Ser Leu
 225 230 235 240
 Phe Asp Gln Val Arg Leu Leu Glu Ser Cys Trp Met Glu Val Leu Met
 245 250 255
 Met Gly Leu Met Trp Arg Ser Ile Asp His Pro Gly Lys Leu Ile Phe
 260 265 270
 Ala Pro Asp Leu Val Leu Asp Arg Asp Glu Gly Lys Cys Val Glu Gly
 275 280 285
 Ile Leu Glu Ile Phe Asp Met Leu Leu Ala Thr Thr Ser Arg Phe Arg
 290 295 300
 Glu Leu Lys Leu Gln His Lys Glu Tyr Leu Cys Val Lys Ala Met Ile
 305 310 315 320
 Leu Leu Asn Ser Ser Met Tyr Pro Leu Val Thr Ala Thr Gln Asp Ala
 325 330 335
 Asp Ser Ser Arg Lys Leu Ala His Leu Leu Asn Ala Val Thr Asp Ala
 340 345 350
 Leu Val Trp Val Ile Ala Lys Ser Gly Ile Ser Ser Gln Gln Gln Ser
 355 360 365
 Met Arg Leu Ala Asn Leu Leu Met Leu Leu Ser His Val Arg His Ala
 370 375 380

(2) INFORMATION FOR SEQ ID NO: 16:

-continued

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 596 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Rattus rattus

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```

Met Thr Met Thr Leu His Thr Lys Ala Ser Gly Met Ala Leu Leu His
1          5          10          15

Gln Ile Gln Gly Asn Glu Leu Glu Pro Leu Asn Arg Pro Gln Leu Lys
          20          25          30

Met Pro Met Glu Arg Ala Leu Gly Glu Val Tyr Val Asp Asn Ser Lys
          35          40          45

Pro Ala Val Phe Asn Tyr Pro Glu Gly Ala Ala Tyr Glu Phe Asn Ala
          50          55          60

Ala Ala Ala Ala Ala Ala Ala Gly Ala Ser Ala Pro Val Tyr Gly Gln
65          70          75          80

Ser Ser Ile Thr Tyr Gly Pro Gly Ser Glu Ala Ala Ala Phe Gly Ala
          85          90          95

Asn Ser Leu Gly Ala Phe Pro Gln Leu Asn Ser Val Ser Pro Ser Pro
          100          105          110

Ile Met Ile Leu His Pro Pro Pro His Val Ser Pro Phe Leu His Pro
          115          120          125

His Gly His Gln Val Pro Tyr Tyr Leu Glu Asn Glu Pro Ser Ala Tyr
          130          135          140

Ala Val Arg Asp Thr Gly Pro Pro Ala Phe Tyr Arg Ser Asn Ser Asp
          145          150          155          160

Asn Arg Arg Gln Asn Gly Arg Glu Arg Leu Ser Ser Ser Ser Glu Lys
          165          170          175

Gly Asn Met Ile Met Glu Ser Ala Lys Glu Thr Arg Tyr Cys Ala Val
          180          185          190

Cys Asn Asp Tyr Ala Ser Gly Tyr His Tyr Gly Val Trp Ser Cys Glu
          195          200          205

Gly Cys Lys Ala Phe Phe Lys Arg Ser Ile Gln Gly His Asn Asp Tyr
          210          215          220

Met Cys Pro Ala Thr Asn Gln Cys Thr Ile Asp Lys Asn Arg Arg Lys
          225          230          235          240

Ser Cys Gln Ala Cys Arg Leu Arg Lys Cys Tyr Glu Val Gly Met Met
          245          250          255

Lys Gly Gly Ile Arg Lys Asp Arg Arg Gly Gly Arg Met Leu Lys His
          260          265          270

Lys Arg Gln Arg Asp Asp Leu Glu Gly Arg Asn Glu Met Gly Thr Ser
          275          280          285

Gly Asp Met Arg Ala Ala Asn Leu Trp Pro Ser Pro Leu Val Ile Lys
          290          295          300

His Thr Lys Lys Asn Ser Pro Ala Leu Ser Leu Thr Ala Asp Gln Met
          305          310          315          320

Val Ser Ala Leu Leu Asp Ala Glu Pro Pro Leu Ile Tyr Ser Glu Tyr
          325          330          335

Asp Pro Ser Arg Pro Phe Ser Glu Ala Ser Met Met Gly Leu Leu Thr
          340          345          350

Asn Leu Ala Asp Arg Glu Leu Val His Met Ile Asn Trp Ala Lys Arg
          355          360          365

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Val Pro Gly Phe Gly Asp Leu Asn Leu His Asp Gln Val His Leu Leu
370 375 380

Glu Cys Ala Trp Leu Glu Ile Leu Met Ile Gly Leu Val Trp Arg Ser
385 390 395 400

Met Glu His Pro Gly Lys Leu Leu Phe Ala Pro Asn Leu Leu Leu Asp
405 410 415

Arg Asn Gln Gly Lys Cys Val Glu Gly Met Val Glu Ile Phe Asp Met
420 425 430

Leu Leu Ala Thr Ser Ser Arg Phe Arg Met Met Asn Leu Gln Gly Glu
435 440 445

Glu Phe Val Cys Leu Lys Ser Ile Ile Leu Leu Asn Ser Gly Val Tyr
450 455 460

Thr Phe Leu Ser Ser Thr Leu Lys Ser Leu Glu Glu Lys Asp His Ile
465 470 475 480

His Arg Val Leu Asp Lys Ile Asn Asp Thr Leu Ile His Leu Met Ala
485 490 495

Lys Ala Gly Leu Thr Leu Gln Gln Gln His Arg Arg Leu Ala Gln Leu
500 505 510

Leu Leu Ile Leu Ser His Ile Arg His Met Ser Asn Lys Gly Met Glu
515 520 525

His Leu Tyr Asn Met Lys Cys Lys Asn Val Val Pro Leu Tyr Asp Leu
530 535 540

Leu Leu Glu Met Leu Asp Ala His Arg Leu His Ala Pro Ala Ser Arg
545 550 555 560

Met Gly Val Pro Pro Glu Glu Pro Ser Gln Ser Gln Leu Thr Thr Thr
565 570 575

Ser Ser Thr Ser Ala His Ser Leu Gln Thr Tyr Tyr Ile Pro Pro Glu
580 585 590

Ala Glu Gly Phe
595

(2) INFORMATION FOR SEQ ID NO: 17:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 591 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Met Thr Met Thr Leu His Thr Lys Ala Ser Gly Met Ala Leu Leu His
1 5 10 15

Gln Ile Gln Gly Asn Glu Leu Glu Pro Leu Asn Arg Pro Gln Leu Lys
20 25 30

Ile Pro Leu Glu Arg Pro Leu Gly Glu Val Tyr Leu Asp Ser Ser Lys
35 40 45

Pro Ala Val Tyr Asn Tyr Pro Glu Gly Ala Ala Tyr Glu Phe Asn Ala
50 55 60

Ala Ala Ala Ala Asn Ala Gln Val Tyr Gly Gln Thr Gly Leu Pro Tyr
65 70 75 80

Gly Pro Gly Ser Glu Ala Ala Ala Phe Gly Ser Asn Gly Leu Gly Gly
85 90 95

Phe Pro Pro Leu Asn Ser Val Ser Pro Ser Pro Ile Met Ile Leu His
100 105 110

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Pro	Pro	Pro	Gln	Leu	Ser	Pro	Phe	Leu	Gln	Pro	His	Gly	Gln	Gln	Val
		115					120					125			
Pro	Tyr	Tyr	Leu	Glu	Asn	Glu	Pro	Ser	Gly	Tyr	Thr	Val	Arg	Glu	Ala
	130					135					140				
Gly	Pro	Pro	Ala	Phe	Tyr	Arg	Pro	Asn	Ser	Asp	Asn	Arg	Arg	Gln	Gly
145					150					155					160
Gly	Arg	Glu	Arg	Leu	Ala	Ser	Thr	Asn	Asp	Lys	Gly	Ser	Met	Ala	Met
				165					170					175	
Glu	Ser	Ala	Lys	Glu	Thr	Arg	Tyr	Cys	Ala	Val	Cys	Asn	Asp	Tyr	Ala
			180					185					190		
Ser	Gly	Tyr	His	Tyr	Gly	Val	Trp	Ser	Cys	Glu	Gly	Cys	Lys	Ala	Phe
		195					200					205			
Phe	Lys	Arg	Ser	Ile	Gln	Gly	His	Asn	Asp	Tyr	Met	Cys	Pro	Ala	Thr
	210					215					220				
Asn	Gln	Cys	Thr	Ile	Asp	Lys	Asn	Arg	Arg	Lys	Ser	Cys	Gln	Ala	Cys
225					230					235					240
Arg	Leu	Arg	Lys	Cys	Tyr	Glu	Val	Gly	Met	Met	Lys	Gly	Gly	Ile	Arg
				245					250					255	
Lys	Asp	Arg	Arg	Gly	Gly	Arg	Met	Leu	Lys	His	Lys	Arg	Gln	Arg	Asp
			260					265					270		
Asp	Gly	Glu	Gly	Arg	Gly	Glu	Val	Gly	Ser	Ala	Gly	Asp	Met	Arg	Ala
		275					280					285			
Ala	Asn	Leu	Trp	Pro	Ser	Pro	Leu	Met	Ile	Lys	Arg	Ser	Lys	Lys	Asn
	290					295					300				
Ser	Leu	Ala	Leu	Ser	Leu	Thr	Ala	Asp	Gln	Met	Val	Ser	Ala	Leu	Leu
305					310					315					320
Asp	Ala	Glu	Pro	Pro	Ile	Leu	Tyr	Ser	Glu	Tyr	Asp	Pro	Thr	Arg	Pro
				325					330					335	
Phe	Ser	Glu	Ala	Ser	Met	Met	Gly	Leu	Leu	Thr	Asn	Leu	Ala	Asp	Arg
			340					345					350		
Glu	Leu	Val	His	Met	Ile	Asn	Trp	Ala	Lys	Arg	Val	Pro	Gly	Phe	Val
		355					360					365			
Asp	Leu	Thr	Leu	His	Asp	Gln	Val	His	Leu	Leu	Glu	Cys	Ala	Trp	Leu
370						375					380				
Glu	Ile	Leu	Met	Ile	Gly	Leu	Val	Trp	Arg	Ser	Met	Glu	His	Pro	Val
385					390					395					400
Lys	Leu	Leu	Phe	Ala	Pro	Asn	Leu	Leu	Leu	Asp	Arg	Asn	Gln	Gly	Lys
			405							410				415	
Cys	Val	Glu	Gly	Met	Val	Glu	Ile	Phe	Asp	Met	Leu	Leu	Ala	Thr	Ser
			420					425					430		
Ser	Arg	Phe	Arg	Met	Met	Asn	Leu	Gln	Gly	Glu	Glu	Phe	Val	Cys	Leu
		435					440					445			
Lys	Ser	Ile	Ile	Leu	Leu	Asn	Ser	Gly	Val	Tyr	Thr	Phe	Leu	Ser	Ser
	450					455					460				
Thr	Leu	Lys	Ser	Leu	Glu	Glu	Lys	Asp	His	Ile	His	Arg	Val	Leu	Asp
465					470					475					480
Lys	Ile	Thr	Asp	Thr	Leu	Ile	His	Leu	Met	Ala	Lys	Ala	Gly	Leu	Thr
				485					490					495	
Leu	Gln	Gln	Gln	His	Gln	Arg	Leu	Ala	Gln	Leu	Leu	Leu	Ile	Leu	Ser
			500					505					510		
His	Ile	Arg	His	Met	Ser	Asn	Lys	Gly	Met	Glu	His	Leu	Tyr	Ser	Met
		515					520					525			
Lys	Cys	Lys	Asn	Val	Val	Pro	Leu	Tyr	Asp	Leu	Leu	Leu	Glu	Met	Leu

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530	535	540													
Asp	Ala	His	Arg	Leu	His	Ala	Pro	Thr	Ser	Arg	Gly	Gly	Ala	Ser	Val
545					550					555					560
Glu	Glu	Thr	Asp	Gln	Ser	His	Leu	Ala	Thr	Ala	Gly	Ser	Thr	Ser	Ser
				565					570					575	
His	Ser	Leu	Gln	Lys	Tyr	Tyr	Ile	Thr	Gly	Glu	Ala	Glu	Gly	Phe	
			580					585					590		

(2) INFORMATION FOR SEQ ID NO: 18:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 518 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Met	Gly	Leu	Glu	Met	Ser	Ser	Lys	Asp	Ser	Pro	Gly	Ser	Leu	Asp	Gly
1				5					10					15	
Arg	Ala	Trp	Glu	Asp	Ala	Gln	Lys	Pro	Gln	Ser	Ala	Trp	Cys	Gly	Gly
			20					25					30		
Arg	Lys	Thr	Arg	Val	Tyr	Ala	Thr	Ser	Ser	Arg	Arg	Ala	Pro	Pro	Ser
		35					40					45			
Glu	Gly	Thr	Arg	Arg	Gly	Gly	Ala	Ala	Arg	Pro	Glu	Glu	Ala	Ala	Glu
	50					55					60				
Glu	Gly	Pro	Pro	Ala	Ala	Pro	Gly	Ser	Leu	Arg	His	Ser	Gly	Pro	Leu
65				70						75					80
Gly	Pro	His	Ala	Cys	Pro	Thr	Ala	Leu	Pro	Glu	Pro	Gln	Val	Thr	Ser
				85					90					95	
Ala	Met	Ser	Ser	Gln	Val	Val	Gly	Ile	Glu	Pro	Leu	Tyr	Ile	Lys	Ala
			100					105						110	
Glu	Pro	Ala	Ser	Pro	Asp	Ser	Pro	Lys	Gly	Ser	Ser	Glu	Thr	Glu	Thr
		115						120					125		
Glu	Pro	Pro	Val	Ala	Leu	Ala	Pro	Gly	Pro	Ala	Pro	Thr	Arg	Cys	Leu
	130					135						140			
Pro	Gly	His	Lys	Glu	Glu	Glu	Asp	Gly	Glu	Gly	Ala	Gly	Pro	Gly	Glu
145					150					155					160
Gln	Gly	Gly	Gly	Lys	Leu	Val	Leu	Ser	Ser	Leu	Pro	Lys	Arg	Leu	Cys
				165					170					175	
Leu	Val	Cys	Gly	Asp	Val	Ala	Ser	Gly	Tyr	His	Tyr	Gly	Val	Ala	Ser
			180					185					190		
Cys	Glu	Ala	Cys	Lys	Ala	Phe	Phe	Lys	Arg	Thr	Ile	Gln	Gly	Ser	Ile
	195						200					205			
Glu	Tyr	Ser	Cys	Pro	Ala	Ser	Asn	Glu	Cys	Glu	Ile	Thr	Lys	Arg	Arg
	210					215						220			
Arg	Lys	Ala	Cys	Gln	Ala	Cys	Arg	Phe	Thr	Lys	Cys	Ile	Arg	Val	Gly
225					230					235					240
Met	Leu	Lys	Glu	Gly	Val	Arg	Leu	Asp	Arg	Val	Arg	Gly	Gly	Arg	Gln
				245					250					255	
Lys	Tyr	Lys	Arg	Arg	Pro	Glu	Val	Asp	Pro	Leu	Pro	Phe	Pro	Gly	Pro
			260					265						270	
Phe	Pro	Ala	Gly	Pro	Leu	Ala	Val	Ala	Gly	Gly	Pro	Arg	Lys	Thr	Ala
	275						280					285			
Ala	Pro	Val	Asn	Ala	Leu	Val	Ser	His	Leu	Leu	Val	Val	Glu	Pro	Glu

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290	295	300
Lys Leu Tyr Ala Met Pro Asp Pro Ala Gly Pro Asp Gly His Leu Pro 305 310 315 320		
Ala Val Ala Thr Leu Cys Asp Leu Phe Asp Arg Glu Ile Val Val Thr 325 330 335		
Ile Ser Trp Ala Lys Ser Ile Pro Gly Phe Ser Ser Leu Ser Leu Ser 340 345 350		
Asp Gln Met Ser Val Leu Gln Ser Val Trp Met Glu Val Leu Val Leu 355 360 365		
Gly Val Ala Gln Arg Ser Leu Pro Leu Gln Asp Glu Leu Ala Phe Ala 370 375 380		
Glu Asp Leu Val Leu Ile Glu Glu Gly Ala Arg Ala Ala Gly Leu Gly 385 390 395 400		
Glu Leu Gly Ala Ala Leu Leu Gln Leu Val Arg Arg Leu Gln Ala Leu 405 410 415		
Arg Leu Glu Arg Glu Glu Tyr Val Leu Leu Lys Ala Leu Ala Leu Ala 420 425 430		
Asn Ser Asp Ser Val His Ile Glu Asp Glu Pro Arg Leu Trp Ser Ser 435 440 445		
Cys Glu Lys Leu Leu His Glu Ala Leu Leu Glu Tyr Glu Ala Gly Arg 450 455 460		
Ala Gly Pro Gly Gly Gly Ala Glu Arg Arg Arg Ala Gly Arg Leu Leu 465 470 475 480		
Leu Thr Leu Pro Leu Leu Arg Gln Thr Ala Gly Lys Val Leu Ala His 485 490 495		
Phe Tyr Gly Val Lys Leu Glu Gly Lys Val Pro Met His Lys Leu Phe 500 505 510		
Leu Glu Met Leu Glu Ala 515		

(2) INFORMATION FOR SEQ ID NO: 19:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 431 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Met Ser Ser Glu Asp Arg His Leu Gly Ser Ser Cys Gly Ser Phe Ile 1 5 10 15
Lys Thr Glu Pro Ser Ser Pro Ser Ser Gly Ile Asp Ala Leu Ser His 20 25 30
His Ser Pro Ser Gly Ser Ser Asp Ala Ser Gly Gly Phe Gly Met Ala 35 40 45
Leu Gly Thr His Ala Asn Gly Leu Asp Ser Pro Pro Met Phe Ala Gly 50 55 60
Ala Gly Leu Gly Gly Asn Pro Cys Arg Lys Ser Tyr Glu Asp Cys Thr 65 70 75 80
Ser Gly Ile Met Glu Asp Ser Ala Ile Lys Cys Glu Tyr Met Leu Asn 85 90 95
Ala Ile Pro Lys Arg Leu Cys Leu Val Cys Gly Asp Ile Ala Ser Gly 100 105 110
Tyr His Tyr Gly Val Ala Ser Cys Glu Ala Cys Lys Ala Phe Phe Lys

-continued

115					120					125					
Arg	Thr	Ile	Gln	Gly	Asn	Ile	Glu	Tyr	Ser	Cys	Pro	Ala	Thr	Asn	Glu
130					135					140					
Cys	Glu	Ile	Thr	Lys	Arg	Arg	Arg	Lys	Ser	Cys	Gln	Ala	Cys	Arg	Phe
145					150					155					160
Met	Lys	Cys	Ile	Lys	Val	Gly	Met	Leu	Lys	Glu	Gly	Val	Arg	Leu	Asp
				165					170					175	
Arg	Val	Arg	Gly	Gly	Arg	Gln	Lys	Tyr	Lys	Arg	Arg	Leu	Asp	Ser	Glu
			180					185					190		
Asn	Ser	Pro	Tyr	Leu	Ser	Leu	Gln	Ile	Ser	Pro	Pro	Ala	Lys	Lys	Pro
		195					200					205			
Leu	Thr	Lys	Ile	Val	Ser	Tyr	Leu	Leu	Val	Ala	Glu	Pro	Asp	Lys	Leu
		210					215					220			
Tyr	Ala	Met	Pro	Pro	Asp	Asp	Val	Pro	Glu	Gly	Asp	Ile	Lys	Ala	Leu
225					230					235					240
Thr	Thr	Leu	Cys	Asp	Leu	Ala	Asp	Arg	Glu	Leu	Val	Phe	Leu	Ile	Ser
				245					250					255	
Trp	Ala	Lys	His	Ile	Pro	Gly	Phe	Ser	Asn	Leu	Thr	Leu	Gly	Asp	Gln
			260					265					270		
Met	Ser	Leu	Leu	Gln	Ser	Ala	Trp	Met	Glu	Ile	Leu	Ile	Leu	Gly	Ile
		275					280					285			
Val	Tyr	Arg	Ser	Leu	Pro	Tyr	Asp	Asp	Lys	Leu	Ala	Tyr	Ala	Glu	Asp
		290					295					300			
Tyr	Ile	Met	Asp	Glu	Glu	His	Ser	Arg	Leu	Val	Gly	Leu	Leu	Glu	Leu
305					310					315					320
Tyr	Arg	Ala	Ile	Leu	Gln	Leu	Val	Arg	Arg	Tyr	Lys	Lys	Leu	Lys	Val
				325					330					335	
Glu	Lys	Glu	Glu	Phe	Val	Met	Leu	Lys	Ala	Ile	Ala	Leu	Ala	Asn	Ser
				340				345					350		
Asp	Ser	Met	Tyr	Ile	Glu	Asn	Leu	Glu	Ala	Val	Gln	Lys	Leu	Gln	Asp
		355					360					365			
Leu	Leu	His	Glu	Ala	Leu	Gln	Asp	Tyr	Glu	Leu	Ser	Gln	Arg	His	Glu
		370					375					380			
Glu	Pro	Arg	Arg	Ala	Gly	Lys	Leu	Leu	Leu	Thr	Leu	Pro	Leu	Leu	Arg
385					390					395					400
Gln	Thr	Ala	Ala	Lys	Ala	Val	Gln	His	Phe	Tyr	Ser	Val	Lys	Leu	Gln
				405					410					415	
Gly	Lys	Val	Pro	Met	His	Lys	Leu	Phe	Leu	Glu	Met	Leu	Glu	Ala	
			420				425						430		

The invention claimed is:

1. An isolated polypeptide displaying the biological activity of ER β , said polypeptide having more than about 95% amino acid sequence identity to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5.

2. An isolated nucleic acid which codes for a polypeptide displaying the biological activity of ER β , said polypeptide having more than about 95% amino acid sequence identity to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5.

3. A recombinant DNA molecule, comprising a DNA sequence which codes for a polypeptide displaying the

55 biological activity of ER β , said polypeptide having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5.

4. A recombinant DNA molecule, comprising a DNA 60 sequence which codes for a polypeptide displaying the biological activity of ER β , said polypeptide having more than about 95% amino acid sequence identity to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5.

5. An expression vector, comprising:

65 (a) a nucleic acid which codes for a polypeptide displaying the biological activity of ER β , said polypeptide

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- having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5; and
- (b) a promoter sequence operably linked to said nucleic acid to allow expression of said nucleic acid and production of ER β .
6. The expression vector of claim 5, wherein said nucleic acid comprises the nucleic acid sequence of SEQ ID NO:1.
7. The expression vector of claim 5, wherein said nucleic acid is clone 29.
8. The expression vector of claim 5, wherein said expression vector is pCMV29.
9. A method for producing ER β , comprising the steps of:
- (a) transforming or transfecting suitable host cells with a recombinant DNA molecule comprising a nucleic acid which codes for a polypeptide displaying the biological activity of ER β , said polypeptide having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5; and
- (b) culturing said host cells under conditions in which said cells express said nucleic acid and produce ER β .
10. The method of claim 9, wherein said recombinant DNA molecule comprises a nucleic acid sequence which

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codes for a polypeptide displaying the biological activity of ER β , said molecule being clone 29.

11. An isolated polypeptide displaying the biological activity of ER β , said polypeptide having more than about 95% amino acid sequence identity to the amino acid sequence of SEQ ID NO:3, and wherein said isolated polypeptide is 485 amino acids in length.

12. An isolated nucleic acid which codes for a polypeptide displaying the biological activity of ER β , said polypeptide having more than about 95% amino acid sequence identity to the amino acid sequence of SEQ ID NO:3, and wherein said polypeptide is 485 amino acids in length.

13. A recombinant DNA molecule, consisting of a DNA sequence which codes for a polypeptide displaying the biological activity of ER β , said polypeptide having more than about 95% amino acid sequence identity to the amino acid sequence of SEQ ID NO:3, and wherein said polypeptide is 485 amino acids in length.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 7,132,261 B2
APPLICATION NO. : 10/278481
DATED : November 7, 2006
INVENTOR(S) : George Kuiper, Eva L. K. Enmark and Jan-Ake Gustafsson

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

(75) On the Title page of the Patent, under the section entitled: "Inventors", the first name of the first named inventor is incorrect. The correct first name of the inventor is --George-- not "Georg" - Kuiper.

Signed and Sealed this

Twentieth Day of March, 2007

A handwritten signature in black ink on a light gray dotted background. The signature reads "Jon W. Dudas" in a cursive style.

JON W. DUDAS

Director of the United States Patent and Trademark Office